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CONTENTS

A Cytological Study of Ascus Development in Ascobolus magnificus Dodge ..... *John L. Wood* 1

The Occurrence of Aneuploidy in Phalaris spp. *A. A. Hanson and Helen D. Hill* 16

Hyphal Systems of Auriscalpium vulgare ..... *M. A. Rogoh* 21

The Growth of Reciprocal Tomato-Tobacco Grafts. *W. Gordon Whaley* 26

New or Noteworthy Species of Tremellales from the Southern Appalachians—II ..... *Lindsey S. Olive* 33

The Requirements of Some Hymenomycetes for Essential Metabolites ..... *Hasan M. Yusef* 42

Studies on Anthocerotales. IV ..... *Johannes Proskauer* 69

An Asplenium Prototype of the Genus Diellia. *Warren H. Wagner, Jr.* 76

Torreya

Notes. Obituary: Francis W. Pennell. Book Reviews. Field Trip Reports 85

Index to American Botanical Literature 102

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BULLETIN OF THE TORREY BOTANICAL CLUB

VOL. 80, NO. 1, pp. 1–15 JANUARY, 1953

A CYTOLOGICAL STUDY OF ASCUS DEVELOPMENT IN *ASCOBOLUS MAGNIFICUS* DODGE[1]

JOHN L. WOOD

For over forty years there has been debate concerning the number of nuclear fusions and reductional divisions in the life cycle of certain members of the Ascomycetes. The solution of problems of this kind is necessary before investigators can arrive at a true insight into the interrelationships and evolutionary tendencies of the group.

Harper (1895) first reported two nuclear fusions in the life cycle of an ascomycete. Later (1905) he reported two fusions in the life cycles of other species. In publishing the results of work done on *Humaria rutilans* Fries, Gwynne-Vaughan (née Fraser) reported in 1908 that there were two nuclear fusions and two reduction divisions in the life cycle of that species. She coined the term "brachymeiosis" for the second reduction division in the ascus. Gwynne-Vaughan (1937) published on *Patella* (*Lachnea*) *melaloma* (Alb. & Schw.) Seaver, reporting two nuclear fusions and two reduction divisions in its life cycle. Olive (1949, 1950), using the propiono-carmine staining technique, demonstrated that there was no evidence of brachymeiosis in *Petella melaloma*. Hirsch (1950), also using a carmine technique and other techniques especially devised for staining chromosomes, stated that there is no evidence that brachymeiosis occurs in *Pyronema confluens* Tul., as was claimed by Gwynne-Vaughan and Williamson (1931).

Olive (1950) gives a more detailed account of the development of the theory of brachymeiosis. Gwynne-Vaughan and all the early workers used sectioned material and most of them stained their material with the iron-alum haematoxylin technique. When the relatively minute chromosomes of the fungi are dehydrated prior to embedding and then treated with a stain such as haematoxylin, which is not at all specific for chromatin, it appears that the work should be viewed with a certain amount of scepticism, unless there has been at least a check made with some other method such as the carmine technique.

In 1912 Dodge described *Ascobolus magnificus*. He later (1920) published a description of its life history. A club-shaped antheridium fuses with the trichogyne tip of a large septate ascogonium. The antheridium and the

[1] Prepared in the Department of Botany, Columbia University, under the direction of Dr. L. S. Olive, this paper is an abridgment of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University, 1951.

The writer is grateful to Dr. B. O. Dodge for several helpful suggestions during the course of this work.

[THE BULLETIN for November–December (79: 427–513) was issued November 18, 1952]

ascogonium arise from different hyphal branches. After plasmogamy a large apothecium is formed. In the hymenial layer asci containing eight ascospores are formed. Dodge reported that single-ascospore cultures never gave rise to apothecia. If two compatible single-ascospore cultures were grown together, however, apothecia were formed. Dodge thus reported the first clear-cut case of heterothallism in the Ascomycetes. He also reported that *Papulospora magnifica* was an imperfect stage of *Ascobolus magnificus*. Dodge did not make a cytological study of this discomycete.

Gwynne-Vaughan and Williamson (1932) also investigated *A. magnificus*. Their work confirmed the findings of Dodge in most aspects. They added to the knowledge of the cultural requirements and morphology of the species and investigated it cytologically. They reported that, for any pair of sex organs, the antheridium arose on a hypha of one strain, and the ascogonium arose on a hypha of a strain of opposite mating type. However, each single-spore mycelium was found to be monoecious, bearing both antheridia and ascogonia. They were the first to report the passage of antheridial nuclei through a septate trichogyne into the ascogonial cell. They claimed that two nuclear fusions and two reduction divisions occurred in the life cycle of *Ascobolus magnificus*. They reported that the haploid chromosome number was four. After fusion in the ascogonium the chromosome number in the ascogenous hyphae was said to be eight. After fusion in the ascus the tetraploid fusion nucleus contained eight bivalent chromosomes. The first division in the ascus was reductional; eight chromosomes went to each pole. The second division was equational and eight chromosomes again passed to each pole. During the third division brachymeiosis was supposed to occur and four chromosomes passed to each pole to give the haploid number in the spores.

Recent work done on some of the Ascomycetes, in which modern techniques were used, has contributed no evidence that brachymeiosis occurs (Olive 1949, 1950; Hirsch 1950). The present investigation was undertaken to study the nuclear behavior in *Ascobolus magnificus* during meiosis and ascus development and to determine whether or not brachymeiosis occurs as claimed by Gwynne-Vaughan and Williamson (1932).

Materials and methods. Ascocarps of *Ascobolus magnificus* appeared on cow dung in moist chambers in the mycology laboratory at Columbia University. The dung had been collected in Greenfield, Massachusetts in November, 1949. During the summer of 1950 the fungus was found fruiting in the pasture where the original dung collection was made. *Ascobolus stercorarius* (*A. furfuraceus*), *Ascophanus carneus*, and an undetermined species of *Saccobolus* were found fruiting together in several of the piles of dung. Dr. Olive obtained the fungus in pure culture by means of the familiar method of inverting an agar plate over the ripe apothecia.

When the fungus was cultured on ordinary media, it did not fruit. On dung decoction agar, with added yeast extract, it formed a few poorly developed apothecia at first and finally ceased to fruit at all. In some cases it fruited well on sterilized cow dung in flasks. In other cases it failed to fruit under the same conditions. During the summer of 1950, a study was made to learn whether a method could be devised to secure apothecia in culture whenever they might be desired.

Early in the culture work single ascospores were isolated after they had germinated. They provided homocaryotic mycelia of the organism. The spores used in the isolations came from the few apothecia which developed from the original culture and from new apothecia collected in the field in the summer of 1950. Single-spore cultures of the fungus failed to produce apothecia at any time and under any conditions. However, when strains were systematically crossed, they were found to be of two types, from the standpoint of sexual compatibility. When compatible strains were crossed upon the correct medium apothecia were formed. This confirmed the observations of Dodge (1920) and of Gwynne-Vaughan and Williamson (1932) that *Ascobolus magnificus* is a heterothallic species.

When apothecia are desired, the best procedure is to use two separate compatible strains as inoculum. Inoculation with material from a mixed culture of the two compatible strains (i.e., cultures in which the strains have been allowed to grow together) has always failed to produce apothecia, in the experience of the writer.

One group of single-spore cultures was designated "A" and another group "B". If only "A" strains or only "B" strains were present in a given culture, no apothecia ever appeared in that culture. Any "A" strain grown together with any "B" strain, under suitable conditions, resulted in the formation of fertile apothecia. Of ten single-spore cultures isolated and tested, four were "A" and six "B".

Ascospores of this species have been reported to be difficult to germinate (Dodge, 1920; Dodge and Seaver, 1946). In the experience of the present writer, when they are not specially treated, the spores usually show less than one per cent germination. Apparently Gwynne-Vaughan and Williamson (1932) had no difficulty in germinating spores of *A. magnificus* sent to them by Dr. Dodge. They reported that some of these spores germinated at 16–18° C; many germinated after incubation at 25° C. They did not state any percentage of germination here, nor did they give any evidence concerning the proportion of ascospores which germinated in later experiments.

In the attempts made during the present investigation, the spores did not respond to heat treatment. Ethyl alcohol (roughly one per cent) added to the medium on which the spores were sown, appeared to increase slightly the percentage of germination. Exposure to fumes of ammonia (suggested by

Dr. B. O. Dodge) also slightly increased germination. At the time work on this problem was discontinued, treatment with about one per cent sodium hydroxide solution for a few minutes, prior to sowing, seemed to promise the best results. The spores swell visibly soon after immersion in the alkali solution.

Investigations now being carried on by Miss Chuan-Chang Yu, a graduate student in mycology at Columbia, will elaborate further upon methods for obtaining good spore germination. It is thought that this fungus can be used advantageously in genetic studies, provided that at least fifty per cent germination of the ascospores can be obtained.

Light is a definite factor in the production of apothecia. Cultures which would otherwise fruit will not produce apothecia if kept in absolute darkness. This observation confirms the findings of Gwynne-Vaughan and Williamson (1932).

Apothecia of *A. magnificus* used as material for the cytological study were grown on a medium consisting of the following:

Casamino acids (Bacto)	0.2 gm.
Sodium carbonate	0.5 gm.
Potassium phosphate (dibasic)	0.5 gm.
Ammonium chloride	0.5 gm.
Zinc sulfate	trace
Ferrous sulfate	trace
Glucose	0.5 gm.
Yeast extract (Difco)	4.0 gm.
Water (distilled)	1000 ml.

The pH of this solution was normally about 8.5 and was adjusted to pH 4.5–5.0 with strong sulfuric acid before sterilization. Twenty milliliters of the adjusted solution were placed in a 250 ml. Erlenmeyer flask. There were also placed in each flask 0.5 gm. of agar and one folded 9 cm. filter paper. The filter paper was folded so that it was about one half inch wide; then the strip thus formed was folded to half its length and inserted in the flask as an inverted V. The flasks were plugged with cotton and sterilized in the autoclave at 15 pounds pressure for twenty minutes. After the agar had cooled and solidified, each flask was inoculated with two separate, compatible strains of *A. magnificus*. The inocula were placed on opposite sides of the medium in the flask.

With the medium and method given above it is possible to obtain all the apothecia desired for study. It would appear that refinements of the basic method given should make possible the successful culture of other coprophilous members of the Ascobolaceae and related groups which do not fruit well on the usual culture media. A species of *Saccobolus* has done well on a

variation of this medium. Further advance in culture methods will be reported upon at a later date by Miss Yu.

Cytological methods. In general, ascocarps were killed, fixed and stained by the modified propiono-carmine procedure mentioned by Olive (1950) and earlier by Godward (1948), in which iron-alum was used as a mordant. The fixing solutions used were a mixture of propionic acid and absolute ethyl alcohol (1:3) or the modified Carnoy solution suggested by Hirsch (1949) for *Hypomyces*. Mordanting was carried out in four per cent iron-alum for one to three hours. Fixation at between two o'clock and four o'clock in the morning yielded the greatest number of division figures in the asci. The material was usually cut into thin slices at the time of fixation. A safety razor blade served well for this purpose. Whether or not fixation is improved by this procedure was not determined, but it was found to be an aid at the time of smearing. It was found helpful to cut away the sterile portions of the larger apothecia before fixation rather than afterwards. When the material had been in the fixative for several hours, it was washed out with several changes of 70% alcohol. The apothecia will keep for months in 70% alcohol. When the material was desired for use, it was run down to water, through a graded series of alcohols, and mordanted in iron-alum. It was then washed in several changes of distilled water, often over night, and used in making smears.

Smears were prepared for study by placing a small portion of the fixed and mordanted material on a slide in a drop of propiono-carmine stain. The stain was prepared according to directions given by Johansen (1940). The iron was left out of the staining solution. A cover slip was placed on the material in the drop of stain and tapped repeatedly and gently with the handle of a pair of forceps or dissecting needle. When the asci were judged to be sufficiently spread out (by observation under a microscope), pressure was applied to the cover slip in order to flatten the asci as much as possible. The most satisfactory method was to invert the slide on a piece of sized cardboard or paper. When filter paper or paper toweling was used, bubbles often gave trouble because too much of the fluid was absorbed by the paper. The inverted slide was pressed down firmly. When the slide was satisfactory, it was ringed with melted glycerine jelly. The ring should be made as quickly as possible after the slide is flattened. Speed saves slides which would otherwise form air bubbles under the cover slip. Sometimes pressure applied to the cover slip while the jelly ring was hardening helped produce a well-flattened preparation. Any debris, such as cotton fibers, under the cover slip made it impossible to obtain a good slide.

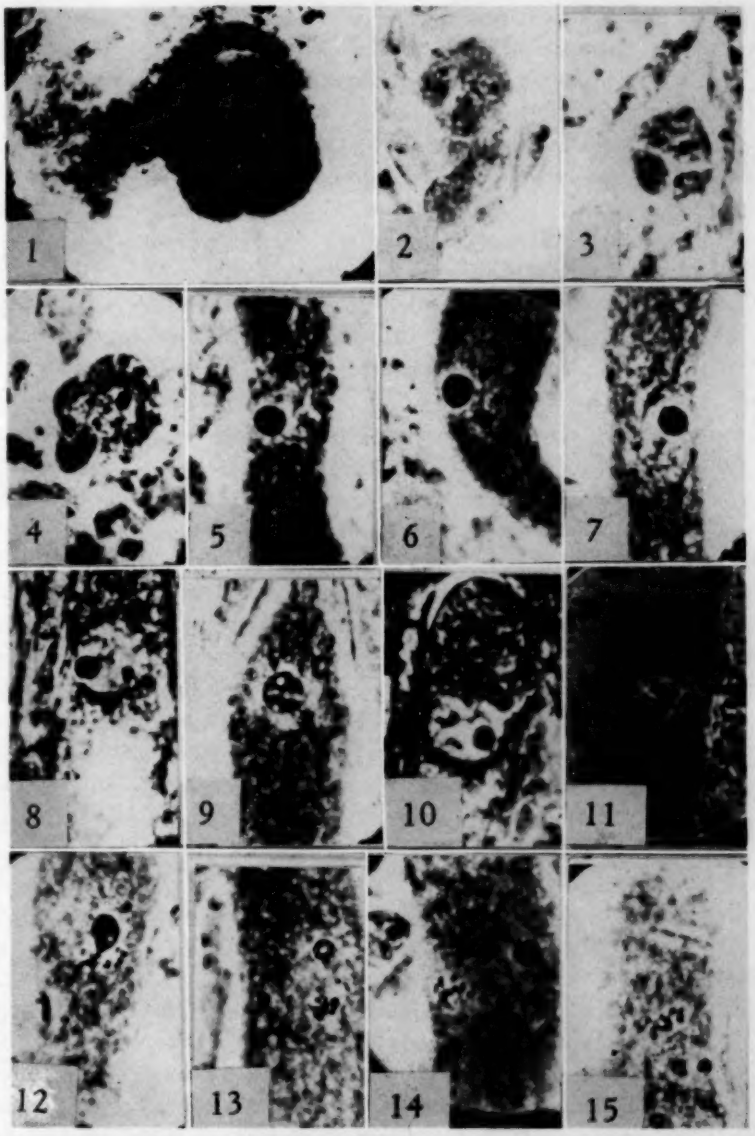
In preparations made from material mordanted in iron-alum, the stain acted rapidly. Chromosomes began to appear well stained within half an hour. Slides which had been kept at room temperature from twelve to

twenty-four hours were in the best condition for study. The writer has made it a practice to keep slides, after the first few hours at room temperature, in the refrigerator until they are to be studied. They will continue to darken while being studied and photographed. If the seal is tight, a condition difficult to attain, the slides will keep for weeks in the refrigerator. At times they become more flattened during this period. Perhaps this is due to drying and shrinking of the jelly ring.

In some cases the writer found that the following modification of the smear technique was effective. The pieces of apothecia were placed in a drop of fluid, preferably the stain or some of the fluid in which the material was standing. A cover slip was placed on the material in the drop and pressed down to partially flatten and spread the layer of asci. The cover slip was then carefully lifted off and the stain added, if it had not already been added. This procedure allowed the stain to penetrate rapidly to all parts of the pressed-out material. After the material had been in the stain with the cover slip off for from three to ten minutes, a clean cover slip was added and the material flattened in the usual manner. This modification resulted in a more even and a more intense staining.

Propiono-carmine stains the chromosomes and the nucleolus. It does not stain the achromatic figure. Because the spindle fibers are not stained, it is a much more reliable and exact technique for counting chromosomes and for studying chromosome morphology than the technique of staining with iron-alum haematoxylin or the triple stain. The iron-alum mordant allows one to see more of the structure of the prophase figures than can be seen in preparations made without the mordant. At all stages of mitosis the chromatin stains more intensely after mordanting than it does when the mordant is omitted.

Optical equipment and graphic methods. Drawings were made with the aid of a camera lucida. With the ordinary microscope a green filter was necessary. During the latter part of the investigation, the author was fortunate in having the use of a phase-contrast microscope. The increase in contrast and sharpness of detail is striking when propiono-carmine smears are examined with this type of equipment. All photomicrographs, except figures 1, 2, 11, 16, and 26, were taken through the phase-contrast microscope. The phase equipment saved much labor because figures which would have been too faintly stained for study under the ordinary microscope were suitable for study when the phase microscope was used. The best combination for photographic work with the phase-contrast microscope was found to be Kodak Contrast Process Panchromatic film, developed for three and one half minutes at 20° C. in Kodak D-19 developer. A Spencer "Dark M" 97x oil immersion objective, N. A. 1.25, was used with standard Spencer mounting and phase equipment.



FIGS. 1–15. Photomicrographs of *Ascobolus magnificus*. FIG. 1. Ascogonium coiled about antheridium. FIG. 2. Prophase of conjugate division in crozier. FIG. 3. Crozier with young, binucleate ascus (penultimate cell). FIG. 4. Apparently nuclear fusion in young ascus. FIG. 5. Prophase I, showing nucleolus and attached chromosome. FIG. 6–10. All prophase I. Nucleolus, expanded chromosomes, and heteropyknotic portions of chromosomes appear (Fig. 8 and 10 are of sectioned material stained with iron-alum haematoxylin.) FIG. 11–15. Metaphase I. Note discharged nucleoli and other chromatic bodies in the cytoplasm. (Fig. 1 × 400; fig. 2, 3, 11, × 1700; fig. 4–10, 12–15 × 1200.)

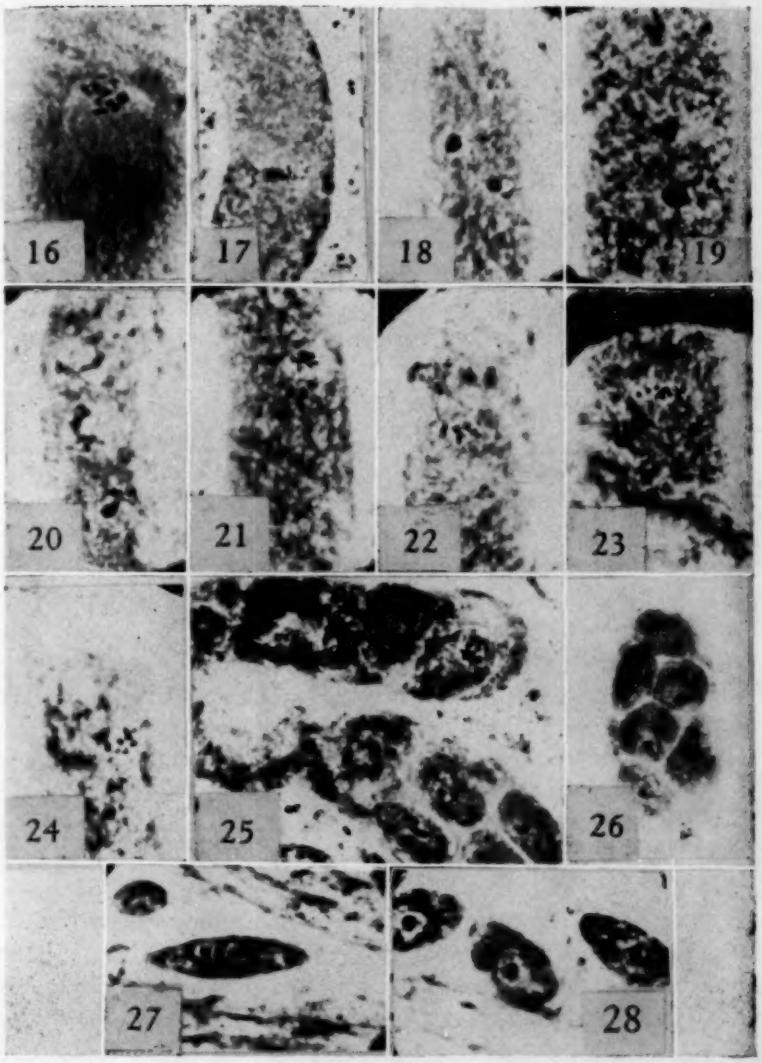
Cytological observations. Four to six days after a culture has been started, sexual organs of the type described by Dodge (1920) and Gwynne-Vaughan and Williamson (1932) appear (figs. 1, 29, 30). The ascogonium is coiled about the antheridium. The asci apparently arise in the usual fashion from binucleate penultimate cells of croziers (figs. 2, 3, 32). It would appear that sometimes nuclear fusion may take place without a final conjugate division (fig. 4). Normally there is a final conjugate division in the crozier (figs. 2, 31). An exact chromosome count was not made in the ascogenous hyphae or croziers.

In the ascus of *Ascobolus magnificus* the nucleolus is a prominent, vacuolate body. It is discharged into the cytoplasm during the course of each nuclear division and reconstructed in each daughter nucleus prior to the next division, as a number of the figures reveal. It appears to be discharged most often just before metaphase, but the exact time may vary somewhat. At the time of discharge it is often associated with a darkly staining spot or granule (figs. 14, 17, 40, 41). The nature of this spot or granule is not fully understood. It is thought that it may be heterochromatin.

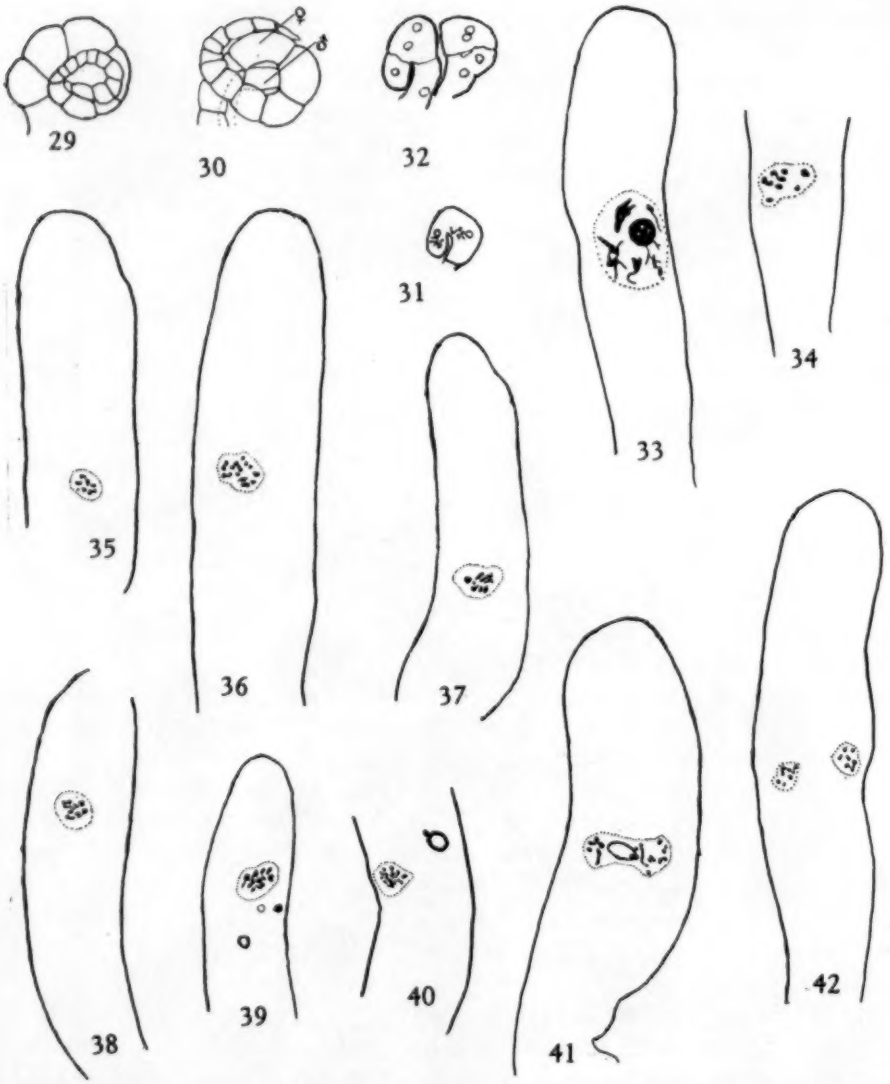
During the prophase the fusion nucleus contains the large nucleolus and several deeply staining masses of material attached to the chromosomes. In most prophase figures one of these masses is conspicuously larger than the others (figs. 6, 7, 8, 33). These are apparently heteropyknotic regions of chromosomes. The material is presumed to be heterochromatin. It is evident that the heavily staining regions are parts of the expanded chromosomes (figs. 7, 10). During meiotic prophase conspicuous extranuclear basophylic particles are seen in the cytoplasm. Their origin is uncertain, although there is a possibility that some of them are heterochromatic bodies discharged from the nucleus (figs. 13–15, 39, 40).

At prophase of the first division in the ascus the chromosomes shorten and thicken. Sometimes the double nature of the strands may be observed. The nucleolus chromosome is frequently seen attached to the nucleolus (fig. 5). At this time and also during prophases of the following divisions the nucleolus chromosome is sometimes attached to the nucleolus until well into metaphase. The same condition has been reported for *Neurospora crassa* (McClintock 1945). At times the portion of the nucleolus to which the chromosome is attached becomes pulled out into a beak (fig. 12). What appears to be a portion (probably heterochromatic) of the nucleolus chromosome is sometimes broken off and remains attached to the nucleolus (fig. 13).

The number of bivalent chromosomes at metaphase I is believed to be eight. There is a possibility that there may be more than eight pairs of chromosomes, but eight was the number most commonly observed (fig. 35, 37). The diploid number of chromosomes in the fusion nucleus of the ascus, therefore, is probably sixteen. This number agrees with the report of



FIGS. 16–28. Photomicrographs of *Ascobolus magnificus*. FIG. 16. Early anaphase I. FIG. 17. Later anaphase I. FIG. 18. Daughter nuclei resulting from first division, showing prominent nucleoli. FIG. 19. Four-nucleate ascus. FIG. 20. Prophase of third division, showing nucleolus chromosome attached to nucleolus in upper nucleus. FIG. 21–23. Stages in anaphase of third division. FIG. 24. Late anaphase of third division. Only one-half of the figure is clearly visible in the photograph. The other complement of chromosomes is mostly out of focus to the upper left of the chromosomes shown. FIG. 25. Young spores in asci. Upper shows the distal spore just being cut out. At least eight chromosomes can be seen. FIG. 26. Young spores in ascus. About eight chromosomes can be seen in the nucleus of the fifth spore from the top of the ascus. FIG. 27. Binucleate spore, showing heterochromatin body near the nucleolus of each nucleus. FIG. 28. Young ascospores, showing nuclei. (Fig. 16, ×1700; fig. 17, 25, ×1000; fig. 18–24, 27, 28, ×1200; fig. 26, ×700.)



FIGS. 29–42. Camera lucida drawings of *Ascobolus magnificus*. FIG. 29. Coiled ascogonium. FIG. 30. Ascogonium coiled around antheridium. FIG. 31. Conjugate division in crozier. FIG. 32. Croziers; only nucleoli drawn in. FIG. 33. Early prophase I; nucleolus and heteropyknotic regions of chromosomes. (Drawn in optical section; the strands of chromatin are all connected in the preparation.) FIG. 34. Late prophase I. FIG. 35. Metaphase I or early anaphase I. FIG. 36. Early anaphase I. FIG. 37. Late prophase or metaphase I. FIG. 38. Early anaphase I; apparently the chromosomes have just split and are starting to separate. FIG. 39. Metaphase or early anaphase I. Nucleolus and other chromatic bodies lie outside the nuclear vacuole. FIG. 40. Anaphase I; about 15 chromosomes are visible. The nucleolus has been expelled from the nucleus and has attached to it, or lying against it, a darkly staining mass, which may be

Gwynne-Vaughan and Williamson (1932) for this stage in ascus development.

Following metaphase I the homologous chromosomes separate. During anaphase I eight univalent chromosomes pass to each spindle pole (figs. 16, 17, 36, 38, 41). The two daughter nuclei with their nucleoli are formed.

At prophase and metaphase of the second division there appear to be eight chromosomes in each nucleus (fig. 42). During anaphase II apparently eight chromosomes pass to each pole. Anaphase II figures were seen the least number of times of any of the stages in the development of the ascus during this investigation. It must be a stage through which the asci pass rapidly. At the end of the second division four nuclei with four prominent vacuolate nucleoli are present in the ascus.

In one preparation the nucleolus chromosome was observed still attached to the nucleolus in late prophase of the third division. The nucleolus was pulled out of shape in a manner similar to that which has been described for prophase I (figs. 20, 43a). The nucleolus chromosome is not the longest chromosome in the set.

During anaphase of the third division the chromosomes split lengthwise and apparently eight chromosomes pass to each pole (figs. 21–24; 46–48). The chromosomes do not all seem to split at the same time and do not pass to the poles simultaneously in two groups, but rather singly. During telophase of the third division spores are cut out in the ascus and each of the eight spores contains a single nucleus. The nucleus in each spore stains well at first and shows a prominent nucleolus and the same heteropyknosis that was exhibited by the primary ascus nucleus (figs. 27, 28). About eight chromosomes can be counted during spore formation (figs. 25, 26, 49–51). Only once during the investigation was a binucleate spore observed. It was approximately twice the size of a normal spore (fig. 27). Both nuclei in the binucleate spore contained heteropyknotic regions.

Discussion. It would appear from the present work that the haploid number of chromosomes in *A. magnificus* is eight. No evidence of a brachymeiotic division was found. The evidence contradicts the claim of Gwynne-Vaughan and Williamson that the haploid number is four and that the fusion nucleus is tetraploid.

Olive (1948) pointed out that modern critical studies have strengthened the view that the nuclei of fungi behave essentially the way those of higher plants do during nuclear division. In view of the present investigation, the

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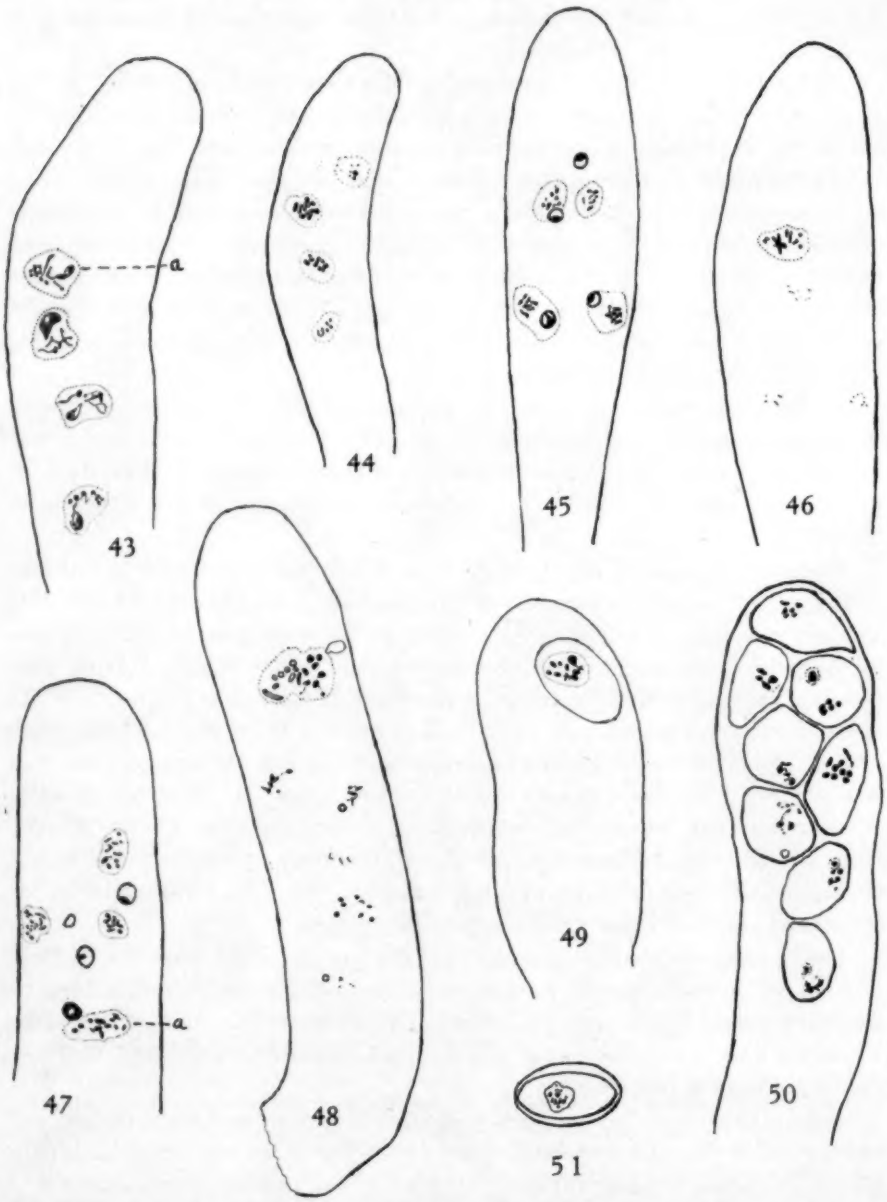
heterochromatin. FIG. 41. Late anaphase I; approximately 16 chromosomes may be counted passing to opposite poles; nucleolus out of focus in center of figure. FIG. 42. Prophase II. (The following drawings and photomicrographs are of the same figures: 30 and 1, 31 and 2, 33 and 7, 38 and 16, 39 and 15, 40 and 14, 41 and 17.) (Fig. 29, 30, × 280; fig. 31, 32, 35, 36, 42, × 1100; fig. 33, 34, 41, × 1200; fig. 37–40, × 700.)



FIGS. 43–51. Camera lucida drawings of *Ascobolus magnificus*. FIG. 43. Prophase of third division; upper nucleus shows nucleolus chromosome attached to nucleolus. FIG. 44. Prophase or early metaphase of third division. FIG. 45. Discharge of nucleoli during third division. FIGS. 46, 47. Anaphase of third division. FIG. 48. Upper figure is of anaphase of third division. 16 chromosomes are shown; one complement is solid, other outlined. Nucleolus has just been discharged. Lower part of ascus was crushed in

comparison of the structure and behavior of fungus nuclei with their structure and behavior in higher plants may be extended.

Masses of chromatic material, similar to those described earlier in this paper, which stain deeply with certain nuclear stains and at times resemble nucleoli, have been reported in higher-plant nuclei. These structures have been described variously as "karyosomes", "chromatin nucleoli", "chromocenters", and "euchromocenters". In some cases at least, they represent definite regions of chromosomes. The term "prochromosome" has been used where these regions seem to be the points at which the prophase chromosomes begin to condense. The regions of the chromosomes which exhibit this densely staining material are said to be "heteropyknotic". Furthermore, the regions in question stain differently at different periods in the development and division of the nucleus. Sometimes the same region may stain intensely at prophase, when it is said to be "positively heteropyknotic", and only lightly at metaphase or after special treatment, such as subjection to cold. In the latter case the term "negatively heteropyknotic" is used. The positively heteropyknotic regions are considered to be those regions of the chromosomes which have not lost their high nucleic acid content after division (White, 1945). It has been found that chromocenters represent parts of chromosomes which have locally preserved their spiral structure.

Gwynne-Vaughan and Williamson (1932) state that two deeply staining bodies, in addition to what appears to be the nucleolus, constantly appear at prophase of the first division in the ascus. They report that these bodies probably represent granules which they describe in the "male" nuclei. Since they claimed that there was a fusion in the ascogonium and a second fusion in the young ascus, they account for the two darkly staining spots as coming from the two "male" nuclei present in the supposedly tetraploid fusion nucleus of the ascus. The present writer believes that they were observing two heteropyknotic regions of a pair of chromosomes. The prophase I figure of Gwynne-Vaughan and Williamson (1932) is much like figures 7 and 10 of the present paper.

Colson (1934) reports darkly staining areas in prophase I nuclei in the ascus of *Neurospora tetrasperma* which may be heterochromatin. McClintock (1945) observed heterochromatic regions of chromosomes of *Neurospora crassa* both in the telophase nuclei and in the resting nuclei of the spores. She did not report heterochromatin in the prophase nuclei.

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smearing and extent of nuclear vacuoles is not clear. FIG. 49. Young spore just being cut out in ascus. Eight chromosomes and what is apparently the new nucleolus are shown. FIG. 50. Eight young spores in an ascus. Note that the fifth from the apex of the ascus shows about 8 chromosomes. (Nuclear membranes not clearly observed.) FIG. 51. Ascospore with thickening wall. (The following drawings and photomicrographs are of the same figures: 43 and 20, 46 and 22, 47 and 23, 48 and 24, 49 and 25, 50 and 26.) (Figs. 43, 45–49, ×1200; fig. ×700; figs. 50, 51, ×1100.)

The presence of at least eight chromosomes in the nuclei makes it difficult to work out the morphology of the individual chromosomes. There is at least one chromosome in the set which is much longer than any of the others, in most of the figures seen. There appears to be a nucleolus-organizing chromosome and this is not the longest chromosome. In *Neurospora crassa* the second longest chromosome is the nucleolus chromosome (McClintock, 1945). In *Patella melaloma* (Olive, 1950) and in *Glomerella* (Wheeler, *et al*, 1948) the longest chromosome has been reported to be the nucleolus chromosome.

Three of the species in the Ascomycetes which Gwynne-Vaughan and her co-workers claimed underwent a second reduction division in the ascus have now been reinvestigated, with newer and more exact cytological techniques. No evidence of brachymeiosis has been found in *Patella melaloma* (Olive, 1949, 1950), *Pyronema confluens* (Hirsch, 1950), or in *Ascobolus magnificus* during the present investigation. The whole idea of brachymeiosis would now seem to be unacceptable.

SUMMARY

1. The theories of double fusion and brachymeiosis in the Ascomycetes and their present status are very briefly outlined.

2. *Ascobolus magnificus* Dodge, collected in Greenfield, Massachusetts, was isolated and grown to maturity in artificial culture.

3. *A. magnificus* is confirmed as a heterothallic species.

4. The cytological development of the ascus was followed with the aid of a modified propiono-carmine staining technique.

5. The phase-contrast microscope was found to be a valuable tool, when used in combination with carmine smears, for cytological studies.

6. Behavior of the nucleolus is described during nuclear divisions in the ascus and the presence of a nucleolus chromosome is reported.

7. Heteropyknosis was observed in the nuclei of the ascus.

8. There are apparently eight bivalent chromosomes at metaphase of the first division in the ascus. Approximately eight chromosomes were observed passing to each pole during anaphase of the third division in the ascus and the same number of chromosomes has been counted in the newly-formed spores.

9. No evidence of brachymeiosis was found during the present investigation of *Ascobolus magnificus*.

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BULLETIN OF THE TORREY BOTANICAL CLUB

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THE OCCURRENCE OF ANEUPLOIDY IN PHALARIS SPP.[1]

A. A. HANSON AND HELEN D. HILL[2]

Chromosome numbers of *Phalaris* spp. have been reported by several investigators (Myers 1947). Information, however, is not available for the Phalarideae on the occurrence or frequency of aneuploidy. In conjunction with an interspecific hybridization program in the Phalarideae, chromosome counts were made on samples representing seven species: *Phalaris arundinacea* L., *P. californica* Hook. and Arn., *P. canariensis* L., *P. coerulescens* Desf., *P. minor* Retz., *P. paradoxa* L. and *P. tuberosa* L. The meiotic behavior of these species was also investigated.

One hundred and twenty-six plant selections of *Phalaris arundinacea* which traced to seed collections made by the Pasture Laboratory were furnished to the writers by the Agronomy Department of The Pennsylvania State College. The 126 selections included 52 plants from ten collections made in north-central Pennsylvania, 19 plants from two collections made in southern New York, and 55 plants from three collections furnished by the Soil Conservation Nursery, Big Flats, New York. A total of 60 plants was examined from two collections made in Washington, and provided by Dr. A. L. Hafenrichter.

The immediate sources of the remaining species were as follows: Soil Conservation Nursery, Pleasanton, California—*Phalaris canariensis* and *P. coerulescens;* Turkey—*Phalaris canariensis;* Portugal—*Phalaris minor;* Soil Conservation Nursery, Lompoc, California—*Phalaris minor;* Denmark and Germany—three samples of *Phalaris paradoxa;* and Beltsville, Maryland—*Phalaris tuberosa.*

Chromosome numbers were determined from paraffin sections of root tips prepared according to the schedule reported by Hill and Myers (1945). Aceto-carmine smears were used in the reexamination of aneuploid plants (Hanson and Oldemeyer 1951). Microsporocytes were collected in acetic alcohol and all data were recorded from fresh aceto-carmine smears.

Experimental results. Frequency of aneuploidy. As shown in Table 1 several aneuploid plants were found in *Phalaris arundinacea.* In the sample studied, 9.14 per cent of the plants varied from the expected chromosome

[1] Contribution No. 111 of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, State College, Pennsylvania, in cooperation with the twelve Northeastern States. The assistance of Mr. Howard L. Hyland in locating *Phalaris* spp. is gratefully acknowledged.

[2] Agent and Assistant Geneticist, respectively.

number of 2n = 28. The frequency of aneuploids in the seven collections that were represented by 12 or more individuals ranged from 0 to 15 per cent.

In *Phalaris minor* two 29 chromosome plants were found in a sample of 60 plants, while no variation was encountered in the two remaining species, *Phalaris californica* and *P. tuberosa,* that have a normal complement of 28 chromosomes. In the latter species only three plants were examined. As might be expected the diploid species, *Phalaris canariensis, P. coerulescens* and *P. paradoxa* did not exhibit any variation in chromosome number.

TABLE 1. *Chromosome numbers determined for seven species of Phalaris.*

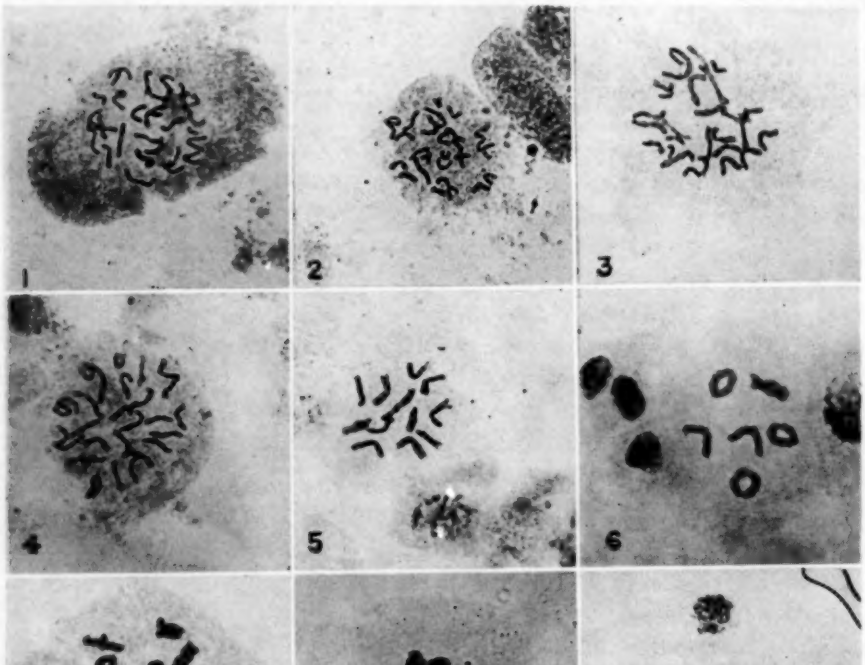
Species	Number of plants	Chromosome Number							
		12	14	27	28	29	30	31	35
P. arundinacea	186			1	169	10	2	1	3
P. californica	30				30				
P. canariensis	60	60							
P. coerulescens	30		30						
P. minor	60				58	2			
P. paradoxa	88		88						
P. tuberosa	3				3				

The chromosomes of *Phalaris arundinacea* are distinguished by marked differences in size. This feature necessitated the reexamination of plants with aneuploid counts in order to avoid improper classification owing to the presence of fragments. Cells with 35 and 29 chromosomes are shown in figures 1 and 2, respectively. In *Phalaris minor* the chromosomes could be separated into two relatively distinct groups of 12 small and 16 large chromosomes (fig. 3). Size differences were also evident at diakinesis, but at least two of the chromosomes appeared to be somewhat intermediate in size (fig. 8). Some variation in chromosomal size was evident in *Phalaris tuberosa,* but size differences were not obvious in the remaining species. *Phalaris californica* (fig. 4) and *P. canariensis* (fig. 5) illustrate this uniformity.

Meiotic behavior. Information on the meiotic behavior of *Phalaris* spp. was obtained in an effort to account for the occurrence of aneuploid plants in *Phalaris arundinacea* and *P. minor.* The average number of half chiasmata per chromosome and the frequency of quartets with micronuclei are summarized in Table 2. In all species, prophase pairing was complete and no multivalent associations were observed. In the samples examined there was no apparent association between chiasma frequency and the frequency of quartets with micronuclei. The average number of half chiasmata per plant ranged from 1.95 to 2.49 in *Phalaris arundiacea,* while quartets with micronuclei per plant varied from 0 to 10.17 per cent. In *Phalaris minor,* the

range in chiasma frequency was 2.31 to 2.55, and the small chromosomes had an average chiasma frequency of 2.12. Quartets with micronuclei varied from 0 to 1.59 per cent.

A portion of the micronuclei observed at telophase II in both *Phalaris arundinacea* and *P. minor* could be fragments, since chromatid bridges and



FIGS. 1–9, ×800. FIG. 1 *Phalaris arundinacea*, 2n = 35. FIG. 2. *P. arundinacea*, 2n = 29. FIG. 3. *P. minor*, 2n = 28. FIG. 4. *P. californica*, 2n = 28. FIG. 5. *P. canariensis*, 2n = 12. FIGS. 6–8. Diakinesis in *P. canariensis*, *P. arundinacea*, and *P. minor*, respectively. FIG. 9. *P. arundinacea*, anaphase bridge and two univalents.

acentric fragments were found in some plants. One plant of *Phalaris arundinacea* (10.17 per cent of the quartets with micronuclei) exhibited an interesting irregularity, in that one or more bivalents failed to become properly oriented at metaphase I. In most cells these bivalents divided and appeared as lagging univalents at anaphase I. A chromatid bridge and two lagging univalents can be observed in figure 9. At least some of the lagging uni-

valents divide, and if both half chromosomes are included in the quartet nuclei the four microspores will have 13, 14, 14 and 15 chromosomes, respectively. No attempts were made at calculating correlations, however, in view of the relatively small samples.

Discussion. Chromosome numbers determined for the seven species agreed with previous reports as summarized by Myers (1947). *Phalaris canariensis, P. coerulescens* and *P. paradoxa* did not exhibit any variation in chromosome numbers, and meiosis was regular as reported by Parthasarathy (1939).

TABLE 2. *Prophase pairing and frequency of micronuclei at telophase II.*

Species	Number of plants	Diakinesis		Quartets	
		Av. No. of Cells	Av. No. of Half X-ta	Av. No. of Cells	Av. per cent with Micronuclei
P. arundinacea	5	18	2.35	173	3.00
P. californica	1	26	2.30	110	0.00
P. canariensis	1	30	2.02	115	0.00
P. coerulescens	2	18	2.30	92	0.00
P. minor	4	20	2.46	112	0.53
P. paradoxa	1	16	2.60	151	0.00
P. tuberosa	1	16	2.59	100	0.00

The lack of multivalent associations suggests that the tetraploid species are allopolyploids. The limitations in accepting multivalent formation as critical evidence for establishing the nature of polyploidy has been emphasized by Nordenskiöld (1945). On the other hand, the existence of distinct size differences in the chromosome complement of *Phalaris minor* would suggest that this species is an amphidiploid. Parthasarathy (1939) found 14 bivalents at diakinesis, but states that an occasional quadrivalent was recognizable in side views of metaphase I. It is possible that the quadrivalents observed by Parthasarathy may have resulted from structural hybridity.

Aneuploidy is a common feature in species which behave cytologically like autopolyploids. Aneuploid plants have been found, however, in diploid *Lolium perenne* L. (Myers, Unpub.) and in *Festuca arundinacea* Schreb. (Peto 1933). Variation in chromosome number was observed in *Phalaris arundinacea* and *P. minor,* but not in *P. californica* or *P. tuberosa.* However, *Phalaris tuberosa* was inadequately sampled. The presence of fragments in some plants of *Phalaris arundinacea* and *P. minor,* together with the wide range in chromosome size necessitated the reexamination of plants with aneuploid counts. The additional chromosomes of the aneuploids reported in Table 1 were within the range of chromosome size encountered in these two species.

The meiotic behavior of *Phalaris arundinacea* together with the chromosome numbers reported in Table 1, suggests that two factors may be contributing to aneuploidy. The 35 chromosome class may have resulted from non-reduction and subsequent crossing of the resulting hexaploid with normal tetraploids, events which would eventually contribute to a wide diversity in chromosome number. Further, the failure in some plants, of one or more chromosome pairs to become properly oriented at metaphase I may result in the addition or loss of one or more chromosomes.

SUMMARY

1. The chromosome numbers of the *Phalaris* spp. investigated agreed with previous reports.

2. The four tetraploid species behaved cytologically like allopolyploids.

3. Aneuploid plants were found in *Phalaris arundinacea* (9 per cent) and *P. minor* (3 per cent).

4. Non-reduction and improper orientation at metaphase I may be contributing to the variation in chromosome numbers.

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HYPHAL SYSTEMS OF AURISCALPIUM VULGARE

M. A. RAGAB

The present study has developed from a desire to introduce more fundamental characters on which to base a new classification of the Hydnaceae. It is hoped that such a study, supplemented by an examination of gross characters, will furnish data which may be useful in presenting an arrangement of species representing more natural genera. The basis for this treatment is one not heretofore used in the systematics of the Hydnaceae, that of the microscopic anatomy of the pileus.

Falck was the first modern mycologist who carried out detailed studies on the anatomy of different Polyporaceae. His most valuable findings have been included in his two monographs on *Lenzites* published in 1909, and on *Merulius* published in 1912. In his monograph on *Merulius,* Falck gave a detailed description of the hyphal systems and their interrelations. He first described the generative hyphae (termed by him "Bildende Faden") which he recognized as giving rise to the hymenial layers. Among other types of hyphae he described in detail the skeletal hyphae (which he termed "Plattenfasern" or "Faserhyphen").

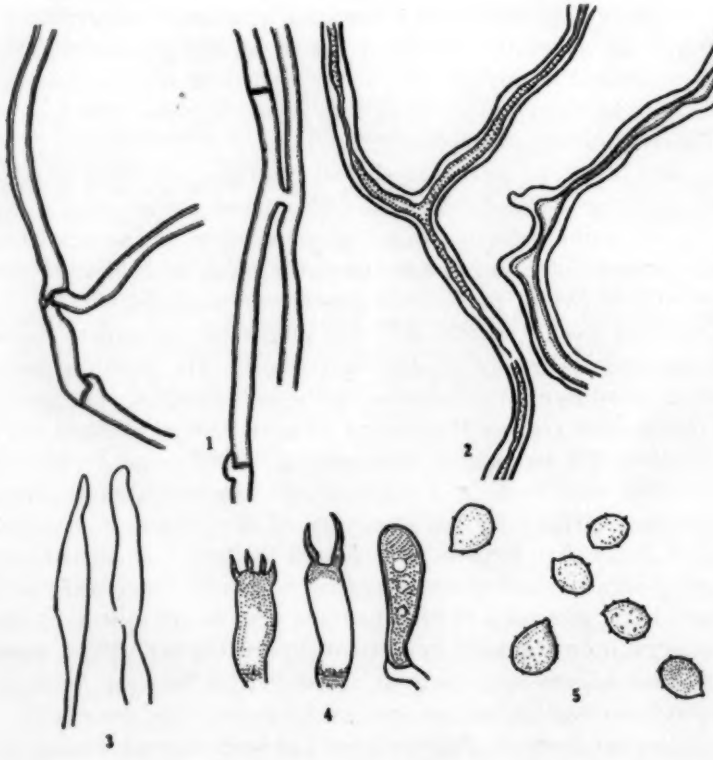
Overholts (1929), by applying the microscopic technique particularly in the Polyporaceae, showed that hyphal characters are often of taxonomic import. The main characters he found of value were: diameter of hyphae; presence or absence of clamp-connections; septation or lack of it; manner and extent of branching; presence or absence of incrustation.

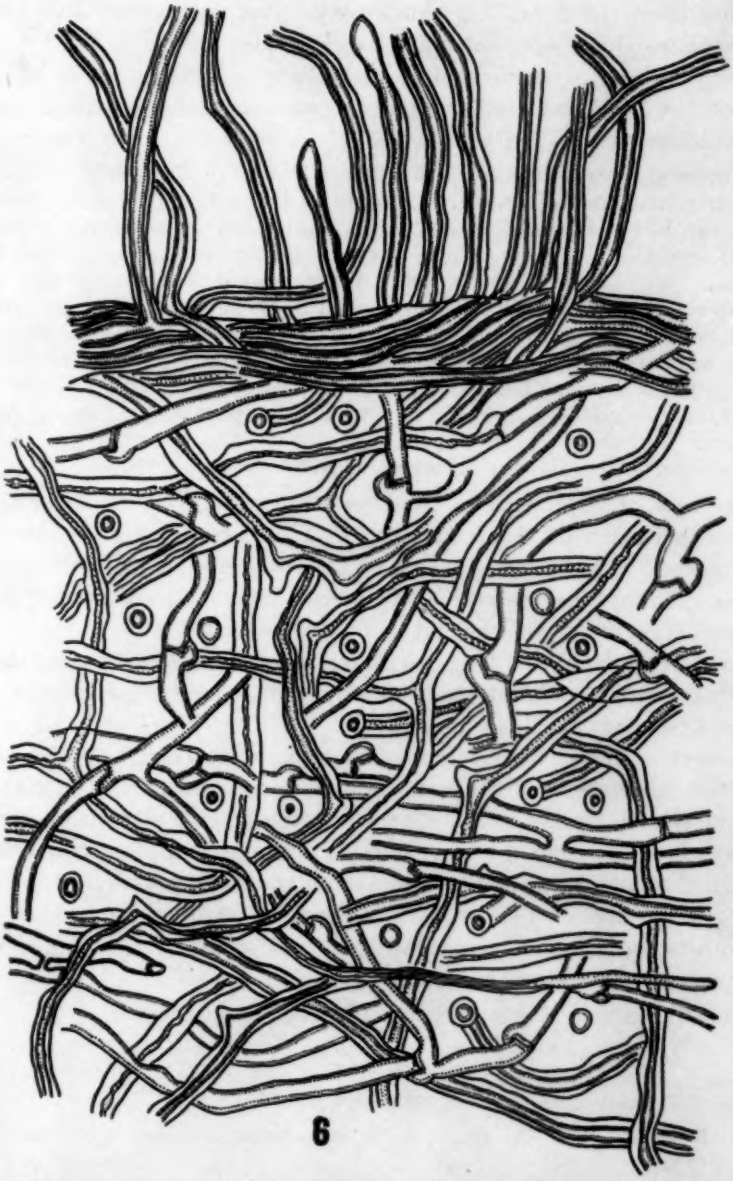
More recently Corner (1932a & b) has published two papers fundamental for the anatomical study of the Polyporaceae. He set up a taxonomic scheme based upon hyphal differences and it is believed it may provide an adequate framework for the Hydnaceae as well. Corner coined the internationally acceptable terms generative and skeletal hyphae for the hyphal types previously met with in Falck's study. He found three important types of hyphae making up the structure of the pileus. A fructification consisting of generative hyphae only is said to have a monomitic system; one having generative and skeletal hyphae is dimitic; and when all three types (generative, skeletal and binding) are present the system is trimitic. In his monograph on *Clavaria* and allied genera Corner (1950) based the classification on microscopic study of the mycelium and the hymenial elements of the fructification and on the development of the fruit body.

The anatomical work on Polyporaceae has been carried a stage further by Reichert and Avizohar (1939), and Cunningham (1947). The first two

authors listed five systems for *Ganoderma lucidum*. In addition to those described by Corner, they recognized palisade and plectenchyma systems. The skeletal hyphae were found by Cunningham to be of two types, "bovista-type" because of their resemblance to capillitium of the genus *Bovista*, and "long type."

Material and methods. This study was carried out on dry herbarium specimens of mature fruit bodies. In microscopical work the system of the hyphae can be followed more readily in free-hand sections teased apart with fine needles under a dissecting microscope. The KOH-Phloxine method remains the most useful technique for dried material. Sections and teased hyphae were placed on a slide, preferably using a binocular, wet with a drop of absolute alcohol which was quickly drained away and replaced with a drop of KOH (3% in water). A small drop of Phloxine was added at one side near the KOH and both were mixed with a needle and a cover slip added. Additional KOH was placed at one side of the mount and excess Phloxine was drawn away from the other side by bibulous paper. Gentle





All figures drawn with camera lucida at a magnification of 1680 diameters and reduced to ×1000. FIG. 1. Generative hyphae with clamp-connections and H-connection. FIG. 2. Skeletal hyphae. FIG. 3. Cystidia. FIG. 4. Basidia: two mature and one immature. FIG. 5. Spores. FIG. 6. Vertical section through a portion of the pileus showing the hyphae parallel to the surface of the pileus and the interwoven interior elements.

tapping upon the cover slip usually separates the hyphae and may yield information which is useful and even valuable.

On the basis of these studies the following description of the species studied has been prepared, with comments on the hyphal organization of the basidiocarp.

AURISCALPIUM VULGARE S. F. Gray, Nat. Arr. Brit. Pl. 1: 650. 1821. Fructification pileate, laterally stipitate; pileus up to 2 cm. wide, horizontal, entire or lobed, cordate to reniform, convex to subplane; surface finely hispid, brown to almost black; margin fimbriate, fertile; stipe vertical, 1–6 cm. long, slender, terete, solid, finely hispid, bulbous-spongy at base, almost black in the upper portion and a lighter shade at the base; substance tough and leathery; spines 0.5–2 mm. long, slender, terete, acute, light brown to grayish, not decurrent; cystidia occasionally present, 20–30 × 3–6 μ, thin-walled, subulate; basidia 15–20 × 5.5–6 μ, clavate, with 2–4 sterigmata; spores 4.5–5.5 × 3.5–4.5 μ, ovoid to subglobose, apiculate, minutely papillose, hyaline.

Growing on coniferous material, usually decaying cones.

Systems of hyphae. Two fundamental hyphal systems are responsible for the formation of the fruit-body of *A. vulgare;* the generative system and the skeletal system. According to the observations of Falck (1912) and Corner (1932b), the generative hyphae are assumed to be the fundamental elements from which the skeletal system is derived.

Generative hyphae: long, cylindrical or somewhat flattened, freely branched, septate, hyaline, thin-walled, clamp-connections present, H-connections frequent, 2–3 μ in diameter.

Skeletal hyphae: these compose the bulk of the fruit body; occasionally branched, aseptate, straight and flexuous, sometimes curled or kinked; the walls internally thickened, so that the lumen is often a mere channel through the center of the hypha, and only the greatly reduced contents stain with phloxine; this opening may be blocked in spots so that at certain levels in the hypha no lumen exists; 2.5–3 μ in diameter; apices thin-walled.

Both types of hyphae are completely interwoven and form a homogeneous structure throughout the pileus.

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THE GROWTH OF RECIPROCAL TOMATO-TOBACCO GRAFTS

W. GORDON WHALEY[1]

The claims of Russian investigators that one component of a grafted plant may "shatter" the hereditary constitution of the other component have served to re-emphasize certain stock-scion relationship problems of rather perennial interest. It has been pointed out repeatedly (Crane 1949, Huxley 1949) that there appear to be numerous deficiencies in the experimental procedures of the Russian investigators who have reported these rather startling graft effects. In any event the effects observed, or many of them at least, are readily explained on the basis of heterozygosity, occasional somatic mutation, or other long recognized and fairly common developments. Wilson and Withner have published (1946) the results of a study of stock-scion relationships in tomatoes designed, in part, as a check upon the work of Avakian and Jastreb (1941). Avakian and Jastreb had reported what they described as graft influences resulting in marked changes in the character of some of the fruits produced on the grafted plants. Wilson and Withner found none of the effects reported.

It is, however, clear from the many descriptions of stock-scion effects reported over several decades that there are definitely influences by which one component of a grafted plant sometimes causes certain modifications of the other component (Roberts 1949). Many such influences have been adequately explained as involving nutritional factors; others have never been studied thoroughly. In no instance, excepting the claims of the Russian investigators, is there any evidence that the modifications are inherited or that the heredity mechanism is in any way involved.

Grafts of tomato and tobacco have provided material in which the site of the formation of nicotine and other alkaloids can be determined and translocation and accumulation of the substances can be followed (Dawson 1942 and 1944). If one considers the Russian investigators' reasoning that the "shattering" of the hereditary constitution of a plant is the result of factors introduced into the environment of the cells and tissues by way of the organisms' nutrition or otherwise, there would seem to be adequate theoretical grounds for assuming that the combination of tobacco and tomato tissues might be more effective in such "shattering" than a combination of tissues of two varieties of tomato. Tomato-tobacco grafts represent more diverse unions than do tomato-tomato grafts.

[1] The author wishes to acknowledge the able technical assistance of Mr. Harry Shute and various members of the physiology section of The Plant Research Institute.

Quite aside from the question of basic modification of one component of the graft by the other, Dawson (1942) has reported certain developments in tomato scions on tobacco stocks which appear to indicate injury to the tomato tissues, possibly because of the accumulation of nicotine.

The work reported here represented an attempt to study certain aspects of growth and development in reciprocal tobacco-tomato grafts in order to determine whether in the particular graft combination used there were any definite stock-scion effects and, if so, whether they were of a transitory character or persisted to maturity.

Materials and methods. The tomato plants used were of University of Texas line 103, derived originally from *Lycopersicon esculentum* Mill., variety yellow plum. The tobacco plants were of a Havana variety of *Nicotiana tabacum* L. supplied by the Massachusetts Agricultural Experiment Station at Amherst, Massachusetts. Several lots of tomato and tobacco seed were planted in flats in the greenhouse during the last two weeks in April. On May 11, healthy, typical plants of both species were transferred to rich soil in three-inch pots. Since the selection at the time of transplanting was rigid the lots of plants were essentially uniform. Just prior to the grafting a second selection was made to eliminate any plants which were then atypical. Grafting was begun on May 17, and completed on May 22. By simple cleft grafts tomato scions were put on tobacco stocks and tobacco scions were put on tomato stocks. Controls were prepared by grafting tomato scions on tomato stocks and tobacco scions on tobacco stocks, using in each case stocks and scions from different original plants. The grafts were tied with raffia and coated with cooled, melted paraffin.

In all, 466 grafts were made. Very few failed to take but in several instances growth subsequent to the grafting was below par. Because of this and some injuries to the plants, approximately 20 percent of the grafted plants were ultimately eliminated from the experiment. When the scions began to show strong growth the remaining plants were transferred, first to six-inch pots and then to nine-inch pots. From the time of grafting all the plants were kept in screened rooms in a lathhouse. To overcome any differences resulting from variation in shading or other factors relating to position, the plants were moved from one position to another in a block pattern every few days. Measured amounts of a supplementary nutrient solution were added to the pots six times during the experiment.

As far as could be determined by direct comparison with greenhouse and field-grown plants growth of the grafted plants appeared to be normal in all respects, excepting only that the partially shaded grafted plants grew somewhat more satisfactorily than unshaded plants in either the greenhouse or the field during the same period.

Beginning early in July, measurements of the plants were begun to pro-

vide a basis for evaluating growth and development. The number of plants measured varied somewhat. The measurements included weight of the plants, which in the case of grafts with tomato scions were pruned to single stalks; stem diameter at 2 cm. above, and 2 cm. below, the graft union and length and width of the six oldest leaves that showed no signs of degeneration. The number of leaves was also recorded. Generally, 10 plants, selected at random from each population, gave an adequate measure of the variation present. For the most part the early measurements were on groups of 10–20 plants. At the later sampling periods the number of control plants measured was somewhat less. At the time each set of measurements was made, a number of plants ranging up to ten of each type were "harvested" for analysis. The plants were divided into samples designated as apical leaf, basal leaf, upper scion, and stock stem samples. The apical leaf samples contained the leaves ranging from the youngest which could be removed readily, leaves only three or four mm. in length, to and including leaves slightly less than half grown, a stage readily determined by measurement of a few mature leaves. The basal leaf samples were composed of the three lower-most fully matured leaves which showed no signs of yellowing or any other indication of deterioration. The upper stem sections consisted of the terminal 10 cm. of the scion stem. The basal stem sections were taken from the stocks and consisted of approximately 8 cm. sections from just below the graft unions.

Nitrogen determinations were made by direct Nesslerization of acid digested mixtures followed by photocolorimetric estimations (Koch and McMeekin 1924). Phosphorus determinations were made by a modification of the Fiske-Subbarow (1925) method following treatment by Wolf's (1944) procedure.

Proteolytic activity was determined by the method of Mounfield, modified in that cystein was used as an activator, thymol sulfonthalein as the indicator, and the initial gelatin concentration was reduced from two percent to one percent. Activity was determined as increase in ml. N/20 NAOH per gm. of fresh weight per 5 ml. of aliquot of digested mixture after a 24 hour digestion at 40° C.

Results. There was an early difference between the height of tobacco on tobacco plants and tobacco on tomato plants (Table 1). At the first sampling period the tobacco on tobacco plants were more than twice the height of the tobacco on tomato plants. By the next sampling period the difference had been considerably reduced and by the third sampling period no significant differences were recorded. There were no significant height differences at maturity. The tomato on tomato and the tomato on tobacco plants differed considerably in height at all the sampling periods. In each instance the tomato on tobacco plants exceeded the tomato on tomato plants in height. Comparison of the number of leaves in these two types of plants

indicates that the difference in height was a result of difference in internode length rather than in the number of internodes formed.

There were no consistent, significant differences between tobacco on tobacco and tobacco on tomato plants as to number of leaves, although the leaf number in the tobacco on tobacco plants was slightly greater at the first two sampling periods. Likewise, there were no leaf number differences between the tomato on tomato and the tomato on tobacco plants.

TABLE 1. *Tobacco on Tobacco (26 plants).*

Date	7/6	7/26	8/16	9/18	10/2
Height (cm.)	19.2 ± 1.6*	30.0 ± 1.9	51.4 ± 2.3	116.4 ± 3.0	123.6 ± 2.9
Number of Leaves	10.0 ± 0.0	16.0 ± 0.0	17.6 ± 0.1	27.3 ± 0.2	36.3 ± 0.8
Leaf Length (cm.)	18.2 ± 0.5	28.4 ± 0.6	31.0 ± 0.6	36.0 ± 0.4	36.2 ± 0.3
Leaf Width (cm.)	9.0 ± 0.4	13.0 ± 0.7	14.5 ± 0.4	18.0 ± 0.5	18.2 ± 0.9
Stem diameter (mm.)					
2 cm. above union	5.5 ± 0.09	6.8 ± 0.3	8.6 ± 0.7	12.8 ± 0.8	12.9 ± 0.6
2 cm. below union	5.3 ± 0.05	6.2 ± 0.5	8.7 ± 0.2	14.2 ± 0.6	14.2 ± 0.5
Nitrogen (percent dry weight)					
Apical leaves	2.76	5.23	5.23	4.53	4.36
Upper scion stem	1.82	3.20	3.16	4.13	3.55
Basal leaves	1.58	2.54	2.61	2.32	1.85
Stock stem	1.46	1.70	1.42	1.11	1.15
Phosphorus (percent dry weight)					
Apical leaves	0.43	0.57	0.61	0.66	0.70
Upper scion stem	0.38	0.46	0.46	0.52	0.56
Basal leaves	0.31	0.33	0.26	0.35	0.28
Stock stem	0.31	0.29	0.22	0.25	0.38
Tobacco on Tomato (161 plants)					
Height (cm.)	7.8 ± 0.8	22.2 ± 1.9	53.3 ± 1.6	112.2 ± 3.0	120.8 ± 2.2
Number of Leaves	9.0 ± 0.0	14.6 ± 0.2	21.0 ± 0.1	27.4 ± 0.3	36.2 ± 0.2
Leaf Length (cm.)	16.0 ± 0.8	28.5 ± 0.8	30.6 ± 0.7	34.8 ± 0.4	37.0 ± 0.4
Leaf Width (cm.)	8.8 ± 0.7	14.5 ± 0.3	14.4 ± 0.5	18.2 ± 0.2	20.2 ± 0.3
Stem diameter (mm.)					
2 cm. above union	6.0 ± 0.08	7.9 ± 0.09	8.3 ± 0.3	9.5 ± 0.6	10.4 ± 0.3
2 cm. below union	5.1 ± 0.03	5.5 ± 0.2	7.5 ± 0.1	8.9 ± 0.2	9.2 ± 0.1
Nitrogen (percent dry weight)					
Apical leaves	2.66	4.73	5.67	5.52	4.48
Upper scion stem	2.03	2.44	4.10	4.31	3.62
Basal leaves	1.54	2.06	2.94	2.15	2.35
Stock stem	1.38	1.60	1.47	1.41	1.56
Phosphorus (percent dry weight)					
Apical leaves	0.39	0.75	0.72	0.71	0.77
Upper scion stem	0.49	0.42	0.46	0.60	0.60
Basal leaves	0.25	0.30	0.32	0.30	0.37
Stock stem	0.36	0.32	0.31	0.26	0.33

* Standard error

TABLE 1 (con't). *Tomato on Tobacco (154 plants).*

Date	7/6	7/26	8/16	9/18	10/2
Height (cm.)	45.0 ± 2.0	87.2 ± 0.9	120.3 ± 0.6	180.3 ± 1.9	210.0 ± 2.6
Number of leaves	11.0 ± 0.2	20.0 ± 0.3	26.2 ± 0.3	38.8 ± 0.4	40.4 ± 0.2
Leaf Length (cm.)	14.6 ± 0.2	20.0 ± 0.1	22.3 ± 0.4	23.2 ± 0.5	24.6 ± 0.4
Leaf Width (cm.)	8.0 ± 0.3	10.6 ± 0.3	11.3 ± 0.2	14.9 ± 0.2	15.4 ± 0.4
Stem diameter (mm.)					
2 cm. above union	4.6 ± 0.2	5.4 ± 0.1	6.6 ± 0.4	7.2 ± 0.2	7.8 ± 0.2
2 cm. below union	6.0 ± 0.3	6.6 ± 0.3	7.6 ± 0.1	8.4 ± 0.2	8.8 ± 0.3
Nitrogen (percent dry weight)					
Apical leaves	3.34	5.50	5.25	4.29	4.52
Upper scion stem	2.58	3.00	3.41	3.01	3.27
Basal leaves	1.73	2.42	1.86	2.06	2.02
Stock stem	1.25	1.45	1.61	1.50	1.50
Phosphorus (percent dry weight)					
Apical leaves	0.57	0.74	0.77	0.68	0.79
Upper scion stem	0.78	0.55	0.56	0.66	0.67
Basal leaves	0.35	0.38	0.37	0.27	0.25
Stock stem	0.37	0.35	0.40	0.31	0.31
	Tomato on Tomato (25 plants)				
Height (cm.)	36.2 ± 0.5	71.0 ± 3.0	97.3 ± 1.8	158.0 ± 1.2	182.0 ± 1.9
Number of Leaves	11.0 ± 0.04	21.0 ± 0.1	24.2 ± 0.4	37.0 ± 0.2	41.2 ± 0.2
Leaf Length (cm.)	22.0 ± 0.6	25.0 ± 0.4	25.2 ± 0.7	24.9 ± 0.3	25.1 ± 0.5
Leaf Width (cm.)	15.3 ± 0.3	14.5 ± 0.2	15.2 ± 0.3	15.1 ± 0.4	14.9 ± 0.2
Stem diameter (mm.)					
2 cm. above union	6.6 ± 0.2	7.6 ± 0.1	7.9 ± 0.08	8.0 ± 0.3	8.3 ± 0.2
2 cm. below union	5.3 ± 0.07	5.6 ± 0.2	5.9 ± 0.1	6.9 ± 0.06	7.2 ± 0.06
Nitrogen (percent dry weight)					
Apical leaves	3.50	4.79	4.86	5.15	
Upper scion stem	2.08	2.96	3.53	4.29	
Basal leaves	2.22	2.82	2.11	2.07	
Stock stem	1.37	1.76	1.90	1.86	
Phosphorus (percent dry weight)					
Apical leaves	0.49	0.58	0.82	0.85	
Upper scion stem	0.63	0.51	0.70	0.84	
Basal leaves	0.30	0.25	0.34	0.28	
Stock stem	0.25	0.22	0.19	0.36	

The leaf length and leaf width measurements recorded provide a means of making at least a general comparison of leaf shapes as well as leaf size. There were no significant variations with respect to either size or shape between tobacco on tobacco and tobacco on tomato plants or, in the later samples, between tomato on tomato and tomato on tobacco plants. At the early sampling periods there were leaf size differences between the tomato on tomato and the tomato on tobacco plants, the leaves of the tomato on

tobacco plants being smaller. The differences would appear to have been the result of somewhat slower expansion of the tomato on tobacco plant leaves.

Stem diameter measurements proved difficult to make because of irregularities in shape of the stems. For this and other reasons the figures on stem diameters tended to vary considerably. Under the conditions of the experiment the tobacco stems attained greater diameters than did the tomato stems. The influence of tomato on tobacco in the grafts was to reduce the size of the tobacco stems whether tomato was used as stock or scion. Used as scion tomato appeared to have a greater effect than when used as stock. There is also a suggestion that tobacco scions promoted greater diameter increase of tomato stocks than did tomato scions.

The measurements and careful observation of the general morphological features of the plants failed to show up any consistent important differences between tobacco growing on tobacco roots or tobacco growing on tomato roots or between tomato growing on tomato roots and tomato growing on tobacco roots. There were clearly some passing differences, observable for varying periods after the actual grafting. The only exceptions were the greater height advantage which the tomato on tobacco plants had over the tomato on tomato plants and the stem diameter effects.

The total nitrogen content of each of the samples was approximately the same in all four graft combinations, suggesting that with respect to this characteristic there are no fundamental quantitative differences between tomato tissue and the tobacco tissues and that no disturbance of the usual situation is brought about by grafting. Likewise, the phosphorus content of the various samples was esentially the same in all of the combinations.

The figures on proteolytic activity have been omitted because they were not complete. The activity of some of the samples was too low to give dependable results. In general, the data show no significant differences among the graft types but do suggest that tomato tissues have somewhat higher proteolytic activity than tobacco tissues.

Discussion. There are on record a number of instances (Roberts 1949) in which the use of different stocks has considerable effect on certain characteristics of the scions. In the experiment reported here no important continuing differences between plants grafted on their own species as root stocks or those grafted on the other species were observed, excepting only the one height difference. There were definitely temporary differences following the grafting, but these differences disappeared more or less rapidly as the plants matured.

Seeds were collected from each of the four types of grafted plants. No especial precautions were taken to prevent cross pollination but inasmuch as the plants were grown in screened rooms from which insects were ex-

cluded, conditions for cross pollination were not favorable. In any event observation of progeny grown from the seed failed to show any individuals modified in any way from the characteristics of the original inbred and tobacco and tomato lines. It can only be concluded that in this instance the two components of the graft are wholly compatible; that, at least to the extent tested, they have only minor morphological effects and no important physiological effects upon each other; and that their association in grafts in no way modifies the heredity mechanism of either.

SUMMARY

1. Tobacco and tomato plants were reciprocally grafted in the late seedling stage.

2. Measurements of height, stem diameter, leaf number, and leaf size showed some temporary differences between grafted plants and controls grafted on stocks of the same species.

3. Except for a height advantage which the tomato on tobacco plants held over the tomato on tomato plants and stem diameter effects, none of the differences observed persisted to maturity.

4. Total nitrogen and phosphorus determinations on apical leaves, basal leaves, upper scion stems and stock stems failed to reveal any differences with respect to the concentration of nitrogen or phosphorus.

5. No modification of genetic constitution was noted.

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NEW OR NOTEWORTHY SPECIES OF TREMELLALES FROM THE SOUTHERN APPALACHIANS—II

LINDSAY S. OLIVE

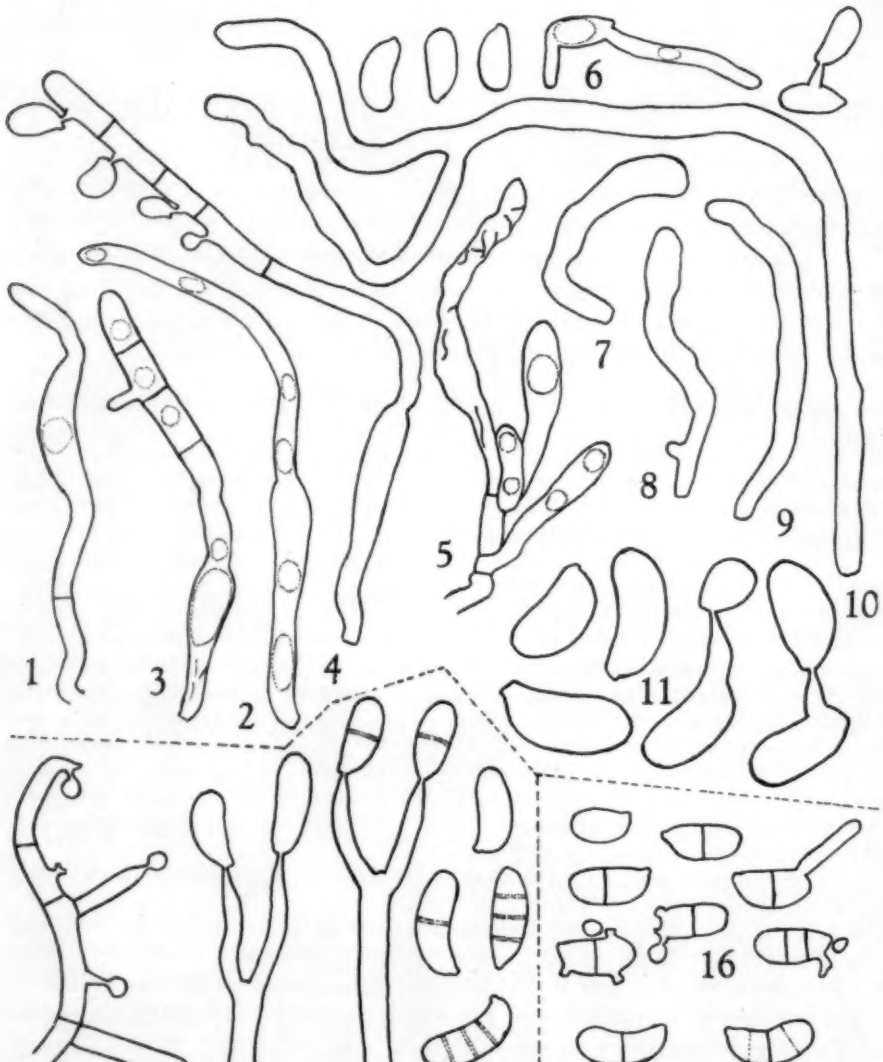
A new species of *Platygloea* and two new species of *Gloeotulasnella* are described. A number of other species seldom collected or not previously reported from the area are briefly described or listed. This is the second in a series of papers, the first of which was published in this journal in 1951.

Platygloea subvestita sp. nov. (figs. 1–11, 18). Ample effusa, subtenuis, sordide hyalina vel alutacea. Gloeocystidia pallide lutea, clavata vel cylindracea vel irregularia, multum elongata, nonnumquam ramosa, 4.7–10.9 × 26.4–210 μ; probasidia clavata, distincta, 7–10.9 × 38.8–63.6 μ; basidiis quadricellularibus, 5–7.8 × 51–91 μ; basidiosporae allantoideae, 5.8–9 × 13.5–21.6 μ. In ligno decorticato frondoso.

Fructifications broadly effused, mostly quite thin, surface tuberculate or nearly smooth in some areas, soft gelatinous to soft waxy gelatinous, sordid hyaline to tan, drying to an invisible film. Hyphae without clamp connections; gloeocystidia clavate or cylindric, sometimes much elongated and occasionally branched, frequently irregular, with yellowish, finely granular to viscous contents, measuring 4.7–10.9 × 26.4–210 μ; probasidia typically clavate, with tapered stalk-like base, distinctly differentiated, arising terminally or laterally on the hyphae, frequently several in a group, measuring 7–10.9 × 38.8–63.6 μ; basidia slender, with 4-celled sporogenous portion, entire basidia measuring 5–7.8 × 51–91 μ, sterigmata short to elongate; basidiospores allantoid, apiculate, 5.8–9 × 13.5–21.6 μ, germinating by repetition or by germ tubes.

Collected on decorticate frondose wood near Highlands, North Carolina, June 22, 1951.

The new species is obviously rather closely related to *P. vestita* Bourd. & Galz., with which it was at first thought to be synonymous. The fructifications are similar in texture and general appearance, and the gloeocystidia of the two species are very much alike in size and shape. However, in *P. vestita* they are hyaline while in our material they are filled with light yellow contents. Furthermore, differentiated probasidia are not described for *P. vestita,* but are quite distinct in the new species. Also both 2- and 4-celled basidia are found in *P. vestita,* but only 4-celled ones have been observed in ours. Bourdot and Galzin (1928) list the size of basidiospores in *P. vestita* as 5–9 × 15–30 μ; Martin (1944) lists the dimensions as 5–7 × 15–25 μ. These measurements are close to the ones which were obtained for *P. subvestita.* However, there is a striking difference in size of basidia. In *P. vestita* they measure 9–10 × 40–50 μ, as contrasted to 5–7.8 × 51–91 μ in *P. subvestita.*



FIGS. 1–11. *Platygloea subvestita*. FIGS. 1 & 2. Germinating probasidia. × 575. FIGS. 3 & 4. Basidia. × 575. FIG. 5. Probasidia and empty basidium. × 575. FIG. 6. Basidiospores. × 575. FIGS. 7–10. Gloeocystidia. × 575. FIG. 11. Basidiospores. × 1040. FIGS. 12–15. *Dacryomitra glossoides*. × 1040. FIG. 12. Conidium-bearing hypha. FIGS. 13 & 14. Basidia producing basidiospores. FIG. 15. Basidiospores. FIGS. 16 & 17. *Dacrymyces minor*. FIG. 16. Basidiospores from fresh collection. × 1040. FIG. 17. Basidiospores from material kept in moist chamber. × 1040.

The writer has examined a single specimen of *P. vestita,* collected by Martin in Iowa, apparently the only collection of the species recorded for North America. It fits very well the description by Bourdot and Galzin, but differs from *P. subvestita* in the details mentioned above.

EXIDIA ALBA (Lloyd) Burt. (fig. 19). The whitish, somewhat flattened corrugate fructifications of this fungus are quite conspicuous. On the basis of its having gloeocystidia in the hymenium, this species was originally described as a *Seismosarca.* However, Martin (1951) has shown that this genus is not valid and that Burt's classification of the fungus is the correct one. The species has been previously collected in the northern United States, Alabama, and the Mississippi Valley. The present collection was obtained along the banks of the Eastatoe River, near Eastatoe, South Carolina, July 16, 1951.

PROTODONTIA UDA Höhn. The species may be recognized by its thin, effuse, waxy-gelatinous fructifications covered with small, conical, spine-like extentions of the hymenium and somewhat resembling an *Odontia.* The basidia are cruciately septate and 4-celled. In our two collections the basidiospores measure 3.9–5.4 × 4.5–8.1 μ.

One small fructification was found growing on an old pine log, while a second collection with fructifications up to 12 cm. in length was obtained along the inner side of an old rotting stump. The latter fructifications were vertically oriented, while the spines protruded horizontally. Both collections were obtained near the Biological Laboratory, Highlands, North Carolina, August 6, 1951. *P. uda* appears to be widespread, but has probably not previously been reported from the southeastern states.

DACRYMYCES MINOR Peck. (figs. 16 & 17). This is one of our most common species of *Dacrymyces.* The particular specimen under discussion, however, showed an unnusual variability in the basidiospores which had not been previously observed by the writer. When first collected and studied in the laboratory it was found to contain basidiospores which were mostly 1-septate, or occasionally 2-septate, and which measured 4–5.9 × 8.2–11.7 μ. However, after the fructifications were kept in a moist chamber for several days, the majority of basidiospores were found to be more narrow and many were 3-septate. These spores measured 3.3–4.5 × 9–13.2 μ. Material kept in the laboratory moist chamber produced basidiospores typical of *D. minor,* while fructifications in their natural habitat were producing what would be considered atypical spores. Whether this was due to a difference in elevation or to other environmental influences is not known. The fungus was collected at an elevation of about 4600 feet and studied at an elevation of about 3900 feet.

Collected on decorticate frondose wood near Teyahalee Bald, Snowbird Mountain Range, North Carolina, June 29, 1951.

DACRYOMITRA GLOSSIODES Bref.? (figs. 12–15, 22). What Brefeld (1888) described as *D. glossoides* is not at all certain. He thought that he had a fungus similar to that described earlier by Persoon (and accepted by Fries) as *Clavaria* (*Calocera*) *glossoides* Pers. It seems likely that Persoon's fungus was a *Dacryomitra,* but his description of it is not sufficient to definitely establish this point. Coker (1928) considers Brefeld's and Persoon's species as synonymous. The fungus described by Coker appears to be the same as ours. There are two specimens (Nos. 33572 and 33573) in the Lloyd Mycological Collections determined by Lloyd as *Dacryomitra glossoides* which are identical with ours. The concept of Coker and of Lloyd is provisionally accepted here, with the hope that future studies will clear up the question as to whether Brefeld's and Persoon's species are synonymous. If not, Persoon's fungus will have to be renamed. A description of our collections follows:

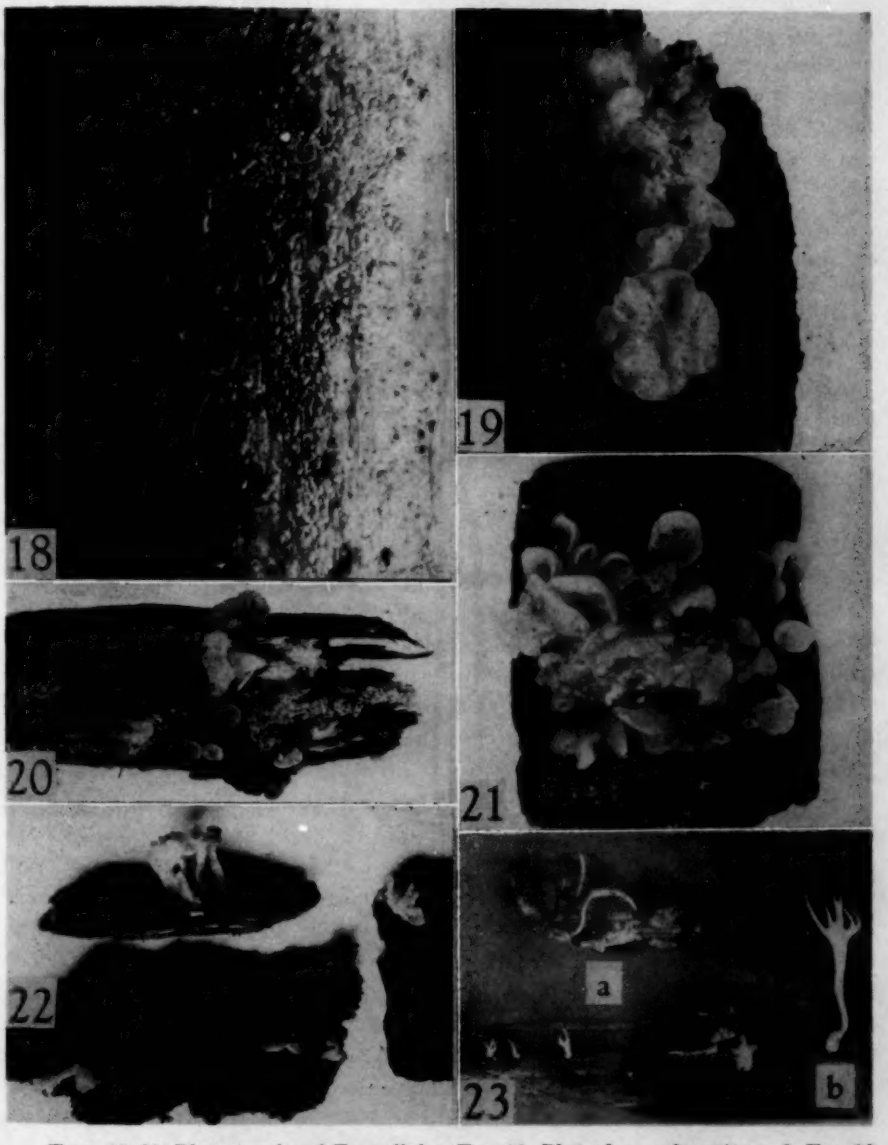
Fructifications firmly gelatinous, pale yellow to orange yellow, 6–12 mm. tall, with stalks (often flattened) 4–7 mm. long and 2.5–7.5 mm. thick, caps smooth to convoluted, hemispherical to flattened in one direction, 4–18 mm. in diam. Hymenium composed primarily of 2-sterigmate basidia and occasional conidium-bearing hyphae, conidia globoid, 1.8–3.2 μ in diam.; basidiospores measuring 3.6–4.5 $\times$ 10–15.3 μ, becoming 1–3-septate.

Collected four times at Highlands, North Carolina and once at Eastatoe, South Carolina, July–August, 1949–1951. On frondose Wood, frequently chestnut.

A collection previously reported by the writer (1951) as *Dacryomitra nuda* (Berk. & Br.) Pat. must be included here. The writer has recently examined five collections from the mycological herbarium at the University of Iowa, which were identified by Brasfield as *D. nuda.* Only two of these (Nos. 57 and 530) fit the description of the species by Berkeley and Broome (1848). Brasfield's description of the species (1938) is based on one of the other three—the only specimen which he had examined at the time. Berkeley and Broome described their fungus as having short, cylindric, white-tomentose stalks and yellow corrugated caps. The fungus on which Brasfield based his description (No. 11095) has a different appearance, particularly in lacking the white tomentum on the stalks (a condition which is very conspicuous in Nos. 57 and 530 when the fructifications are dry). It appears evident that Brasfield's concept of *D. nuda* is based upon his observations on another species. None of our collections from the southern Appalachians are of this type.

FEMSJONIA PEZIZAEFORMIS (Lév.) Karst. (fig. 20). Martin reports this species as being widespread in the cooler parts of the northern hemisphere. The writer (1947) found it once previously in Georgia. This appears to be the second report of its occurrence in the south. The fungus is distinguished by its yellow to orange yellow disc-shaped or pezizoid fructifications that are

sessile or short-stalked. The basidiospores are curved cylindric and become 1–7-septate. Collected on corticate frondose limbs, near Eastatoe, South Carolina, July 25, 1951.



FIGS. 18–23. Photographs of Tremellales. FIG. 18. *Platygloea subvestita*, × 2. FIG. 19. *Exidia alba*. × 1½. FIG. 20. *Femsjonia pezizaeformis* (slightly enlarged). FIG. 21. *Guepiniopsis torta*, × 2. FIG. 22. *Dacryomitra glossoides*, × ⅔. FIG. 23. *Calocera cornea* (a) and *C. viscosa* (b), × ⅔.

GUENINIOPSIS TORTA (Fr.) Pat. (figs. 21 & 24–28). It is obvious from the original descriptions by Persoon (1801) and by Tulasne (1853) that *Guepiniopsis merulinus* (Pers.) Pat. and *Guepinia peziza* Tul., respectively, are synonymous with *Guepiniopsis torta*, as Brasfield (1938) has noted. It is an easily recognized species and is readily distinguishable from other members of the genus. Nevertheless, the writer has found considerable confusion concerning this species both in herbaria and in the literature. A recent collection obtained by the writer, which fits well the earlier descriptions of the species, is described as follows:

Fructifications closely gregarious, mostly orange yellow, firmly gelatinous, regularly or irregularly pezizoid, short-stalked, both stalks and caps frequently coalescing, externally sulcate-ribbed, up to 6 mm. tall, caps 2.5–9 mm. broad; drying brilliant orange to reddish orange. Internal hyphae not gelatinizing readily, clamp connections absent; cortical cells vesicular to elongate, often rough-walled, typically in simple or branching chains, thin-walled or with thick gelatinizing walls; basidia 2-sterigmate; basidiospores 3.8–6.3 × 10–14 (– 17) μ, becoming inconspicuously 1–3-spetate, budding out small conidia.

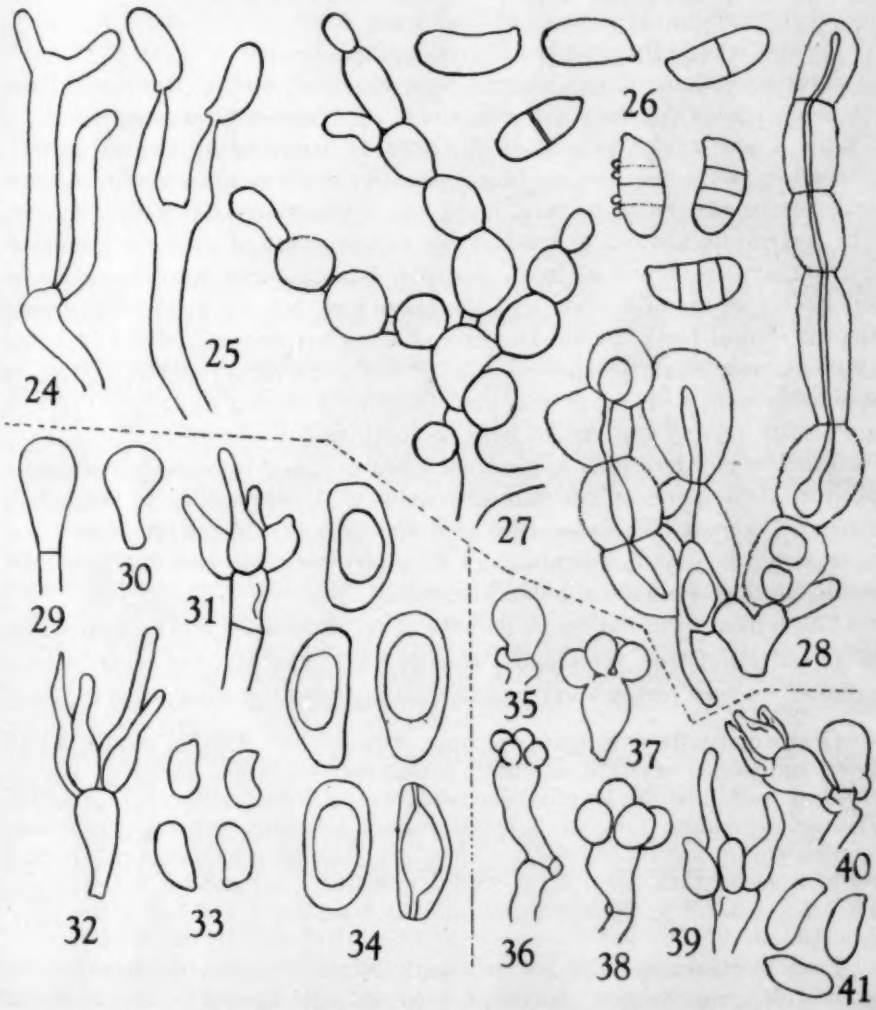
Growing from side of oak stump with the concave surfaces of fructifications facing downwards; Highlands, North Carolina, July 7, 1949. Also collected twice in July, 1949.

The features which particularly distinguish this species from all others are the catenulate cortical cells and the conspicuous striations in the exterior of the fructifications. These characteristics are particularly wel[1] illustrated by Tulasne (1872). Tulasne observed the chains of cortical cells, which are frequently separable, and considered the possibility that they were conidia. On the outside of the cup, these cells are mostly thin-walled (fig. 27), but further down on the stalk they have very thick gelatinous walls and narrow lumens (fig. 28).

Specimens in the mycological collections at the University of Iowa identified by Brasfield as *G. torta* are quite different from ours. One of the collections on which Brasfield (1938) bases his discussion of the species and which he used for his drawings of the peridial hairs has smaller, duller fructifications without the conspicuous external striations. Furthermore, the peridial hairs consist primarily of single clavate or cylindrical cells with thickened walls. This material can certainly not be considered representative of *G. torta*. The writer has also collected a specimen which resembles most of the material identified by Brasfield as *G. torta,* but has not yet succeeded in identifying it. It appears to be a deminuitive *Guepiniopsis* in which the stalks are very short or almost lacking and in which the cortical hairs are not as abundant or as well modified as in typical members of the genus.

CALOCERA VISCOSA Fr. and C. CORNEA Fr. (fig. 23). *C. viscosa* (fig. 23b) is said to be widespread, but it has seldom been collected in this country and

it does not appear to be common in North America. It is distinguished from the much more common *C. cornea* (fig. 23a) by its larger size and the more profuse branching of its orange yellow fructifications. The basidiospores are very nearly the same size in both species, and in both they become 1-septate. The single fructification in our collection of *C. viscosa* was 3.2 cm.



FIGS. 24–28. *Guepiniopsis torta.* FIGS. 24 & 25. Basidia. FIG. 26. Basidiospores. FIG. 27. Chains of vesicular cells from exterior of cap. FIG. 28. Chains of vesicular cells from stalk. FIGS. 29–34. *Gloeotulasnella aggregata.* FIGS. 29 & 30. Probasidia. FIGS. 31 & 32. Basidia with epibasidia. FIG. 33. Basidiospores. FIG. 34. Gloeocystidia. FIGS. 35–41. *Gloeotulasnella caroliniana.* FIG. 35. Probasidium. FIGS. 36–40. Stages in development of basidia. FIG. 41. Basidiospores. (All figures × 1040.)

tall. Its spores measured 3.5–5 × 8.4–14 μ. The particular collection of *C. cornea* which is illustrated here had simple or sparingly branched fructifications, some of which were unusually long for this species. They varied from 3.5–25 mm. in length. The basidiospores measured 3.2–4.2 × 8.4–14 μ.

The fructification of *C. viscosa* was found by Dr. Leland Shanor on an old stump at Highlands, North Carolina, August 4, 1951. *C. cornea* is collected throughout the summer at Highlands.

TULASNELLA VIOLEA (Quél.) Bourd. & Galz.

Two collections of this species were obtained, one of them with thin lilaceous, somewhat waxy fructifications, with spores measuring 4.1–4.6 × 5.6–6.6 μ, and rather typical of *T. violea* as described by Rogers (1933). The other had a conspicuous lilaceous color, but was considerably thicker and soft gelatinous in texture, more like a *Gloeotulsnella* in this respect. The hymenium showed a considerable development of vertical basidium-bearing hyphae, much as in *G. pinicola*. Basidiospores were found to be subglobose to broadly ovate and the spore size, 5.5–7.5 × 7.5–10 μ, larger than is typical for *T. violea*. However, Rogers has emphasized the fact that this is a very variable species, and he has reduced numerous species to synonymy with it. There is some good evidence here that our two collections are closely related and should both be considered forms of *T. violea*. They both have a powdery pink appearance when dry, and in both the epibasidia produce their spores on subulate extensions of the epibasidia. If the second collection were a *Gloeotulasnella,* as it was once considered, the fructifications would be expected to dry to a thin vernicose film and the epibasidia would produce elongate tubular filaments.

The typical form was found on bark of frondose wood and on sporocarps of an old *Polyporus, Highlands,* June 9, 1951. The atypical material was collected on very rotten wood, near Eastatoe, South Carolina, July 16, 1951.

Gloeotulasnella aggregata sp. nov. (figs. 29–34). Effusa, tenuis, ad 10 cm. longitudine, sordida, molliter gelatinosa, superficie sublaevi vel irregulari undulataque. Hyphae enodosae, 1–6 μ diam.; gloeocystidia multipliciter aggregata, breviter subcylindracea, lateribus rectis vel curvulis, hyalina vel luteuscula, 6.1–9.1 × 13.6 × 20 μ; basidia plerumque pyriformia vel globoso-capitata, 5.4–7.2 × 9–15.3 μ, epibasidiis subglobosis vel oblongis, 4.5–5.9 × 6.3–7.3 μ; basidiosporae allantoideae, 3–3.8 × 6.7–9 μ. In ligno decorticato.

Fructifications up to 10 cm. in length, effuse, thin, surface nearly even to irregular and undulate, sordid in color, soft gelatinous, drying to an almost invisible film. Hyphae without clamp connections, 1–6 μ diam.; gloeocystidia short cylindrical usually narrowing somewhat towards one or both ends, sides straight or convex, contents rather coarsly granular, hyaline or nearly so, with thick gelatinizing walls, measuring 6.1–9.1 × 13.6–20 μ; basidia mostly pyriform to globose-capitate, 5.4–7.2 × 9–15.3 μ; epibasidia

subglobose to oblong, 4.5–5.9 × 6.3–7.3 μ, producing elongate spore-bearing filaments; basidiospores allantoid, apiculate, 3–3.8 × 6.7–9 μ.

Growing on rotting decorticate wood, near Highlands, North Carolina, July 30, 1951.

The species name is derived from the tendency of the gloeocystidia to aggregate into groups which can be detected even with a high-powered hand lens. If some of the material is mounted in potassium hydroxide for an hour or so or is left in a moist chamber overnight, the thick walls of the gloeocystidia frequently gelatinize completely and the entire structures disintegrate.

The closest related species to *G. aggregata* is probably *G. rogersii* Olive (in press), which also has gloeocystidia and allantoid spores. No other species of *Gloeotulasnella* with allantoid spores has been described. *G. rogersii* differs from the new species particularly in having distinctly yellow gloeocystidia with much thinner walls. Furthermore, the gloeocystidia of the former are much more variable in size and shape and do not aggregate into clusters. Other less significant differences have also been observed.

Gloeotulasnella caroliniana sp. nov. (figs. 35–41). Tenuis, effusa, molliter gelatinosa, griseo-hyalina, vel isabellina. Hyphae 1.7–6.3 μ diam., zygodesmatibus ornatae; basidia obovata vel pyriformia, 5.4–8.6 × 9–14.4 μ; epibasidiis plerumque subglobosis vel obovatis, 4–5.4 × 5.5–7.6 μ; basidiosporae cylindraceae, curvulae, 3.1–3.8 × 8.5–10.4 μ. In ramis decorticatis frondosis.

Forming thin, soft gelatinous layers on the substrate, surface appearing uneven when magnified, color grayish hyaline to tan, drying to a very inconspicuous to invisible film. Hyphae 1.7–6.3 μ diam., clamp connections present; no gloeocystidia; basidia ranging from obovate to pyriform, frequently with short stalk-like base, measuring 5.4–8.6 × 9–14.4 μ; epibasidia mostly subglobose to obovate, 4–5.4 × 5.5–7.6 μ, producing filamentous sporogenous filaments; basidiospores cylindric, slightly curved, apiculate, 3.1–3.8 × 8.5–10.4 μ.

Growing on decorticate frondose limbs, near Wayah Bald, North Carolina, June 29, 1951.

The shape of the basidiospores, which are very nearly allantoid, distinguishes this species from all other species of *Gloeotulasnella* lacking gloeocystidia.

The fungi described in this paper were collected during the writer's residence at the Highlands Biological Station. Type material of the new species has been deposited in the Mycological Collections at the New York Botanical Garden. The writer is grateful to Dr. D. P. Rogers for his preparation of the Latin descriptions.

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THE REQUIREMENTS OF SOME HYMENOMYCETES FOR ESSENTIAL METABOLITES

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In a previous investigation (Robbins 1950) from this laboratory, some fungi including species of *Polyporus* and *Fomes* were found to grow poorly or not at all on a basal medium of asparagine, dextrose and mineral salts. The poor growth of some of these appeared to be caused by a lack of vitamins in the basal medium; others responded to the addition of vitamin-free casein hydrolysate to the basal medium; some required the addition of both vitamins and casein hydrolysate; a few grew well on malt extract but poorly on the basal medium supplemented with both vitamins and casein hydrolysate.

The present investigation was undertaken to define more clearly the nutritional requirements of some of the species and strains included in the earlier survey.

Materials and methods. The cultures of fungi used, with one exception, were originally received from Ross W. Davidson and maintained on malt extract agar. The numbers given are those used by Davidson. We are indebted to Dr. Dow V. Baxter for a strain of *Polyporus texanus*, number B 734 in our collection.

Both liquid media and media solidified with purified agar (Robbins and Ma 1945) were used. Unless otherwise stated, the following basal solution was employed:

Dextrose	50 gm.
KH_2PO_4	1.5 gm.
$MgSO_4 \cdot 7H_2O$	0.5 gm.
Asparagine	2.0 gm.
Trace elements	0.5 ml. (equivalent in p.p.m. to 0.005 B, 0.02 Cu, 0.10 Fe, 0.01 Ga, 0.01 Mn, 0.01 Mo and 0.09 Zn prepared from H_3BO_3 57 mg., $CuSO_4 \cdot 5H_2O$ 157 mg., $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ 1404 mg., $Ga_2(SO_4)_3$ $18H_2O$ 108 mg., $MnSO_4 \cdot 4H_2O$ 81 mg., Acid molybdic (85%) 36 mg., $ZnSO_4 \cdot 7H_2O$ 790 mg., distilled water to make 1 liter of solution.)
Distilled water to make 1 liter.	

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[2] The writer expresses his gratitude to the Faculty of Science, Farouk I University, Alexandria, Egypt, for granting him a scholarship and study-leave which made this research possible. His thanks are extended also to W. J. Robbins, M. Anchel, A. Hervey, F. Kavanagh and I. M. McVeigh for advice and assistance.

Vitamin-free casein hydrolysate, C.P. dextrose and asparagine purified by treatment with Norit A and recrystallization from redistilled alcohol were used. Other chemicals were reagent grade.

All glassware was Pyrex brand and was cleaned with chromic acid cleaning mixture and thoroughly rinsed with tap and distilled water.

The pH value of all media before and after growth was measured electrometrically. Unless otherwise stated, all media were adjusted with NaOH so that the hydrogen-ion concentration after sterilization was about pH 5.0. Media were sterilized by autoclaving at 15 pounds pressure for 20 minutes. Bleached cotton was used for plugs.

For liquid cultures 25 ml. of media were placed in 125 ml. Erlenmeyer flasks, and for solid media about 8 ml. were used per tube.

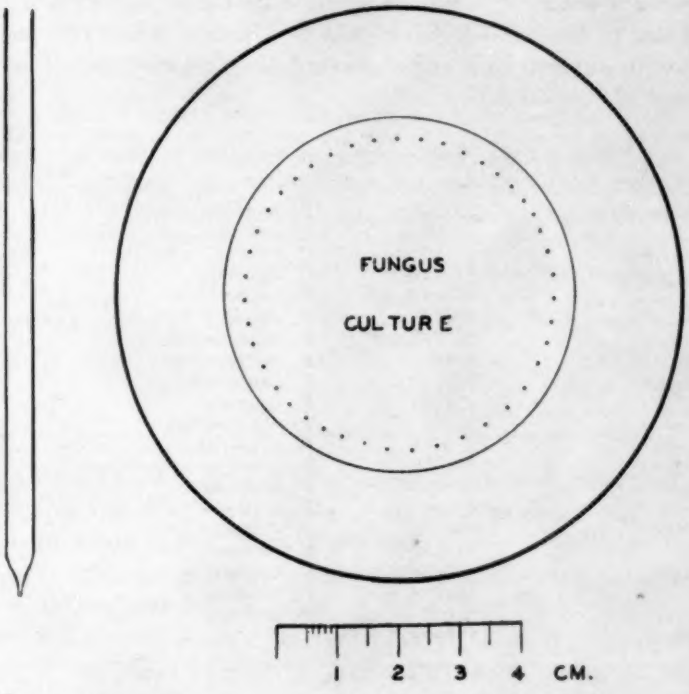
Inoculations were made by transferring from plate cultures on malt extract agar small discs about 1 mm. in diameter for flask cultures and 0.5 mm. for tube cultures. These discs were cut in the advancing margin of the culture with glass droppers having internal pores of the mentioned diameters (fig. 1). The glass droppers were sterilized by immersing in alcohol for a few minutes, draining and flaming the remaining alcohol. Almost all of the fungus-free portions of the cut discs were removed to minimize the carrying over of growth factors.

Cultures were incubated at 25° C. For liquid cultures, growth was measured in terms of dry weight (average of three determinations) by filtering the mycelium, after coagulation with alcohol, onto a disc of filter paper in a weighed perforated Gooch crucible, washing with warm distilled water and drying at 100° C. In any single experiment mycelia were usually harvested when the mycelium completely covered the medium in one or more series of cultures. For tube cultures, the diameter and density of growth were taken into consideration and growth was expressed in comparative values. Differences in growth that were not detected by weight could be observed in tube cultures. Furthermore, observations could be more easily repeated at intervals on tube cultures.

Vitamin requirements. Thirty-four species of *Polyporus* (plus duplicate isolations of three species and one variety of a fourth) and three species of *Fomes*, all of which had been found previously in this laboratory to evidence vitamin deficiencies, were investigated.

Each of the fungi was grown in triplicate in test tubes on the basal agar medium, the basal medium supplemented with eight vitamins and on a series of media from which one of the vitamins in turn was omitted but the other seven included. Thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, niacin, calcium pantothenate and *p*-aminobenzoic acid were added at the rate of 10 mμ moles per tube containing 8 ml. of medium; biotin at 0.05 μg. and inositol at 10,000 mμ moles per tube. For *Fomes fraxinophilus*,

Polyporus guttulatus and *P. immitus*, the basal solution was supplemented with 0.04 per cent casein hydrolysate; for *P. schweinitzii* casein hydrolysate



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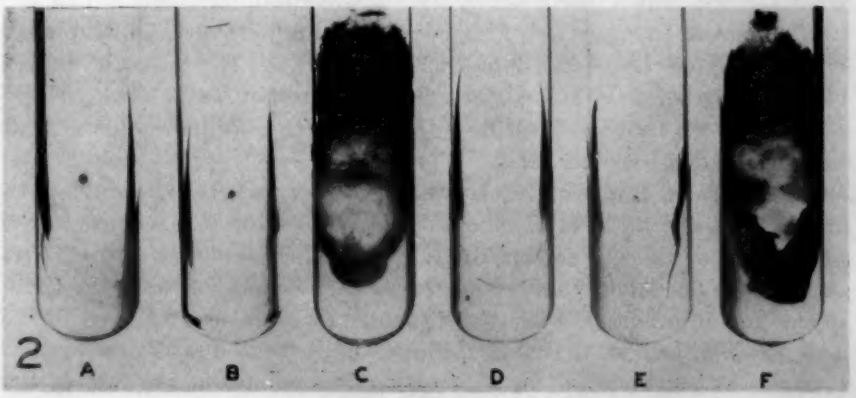


FIG. 1. Preparation of inoculum. To the left, glass dropper to cut equal discs (represented by dots in dish to the right) near advancing edge of fungus culture. FIG. 2. Response to thiamine. Growth for 12 days of *Polyporus velutinus* (A, B, C) and *P. pubescens* (D, E, F) on basal agar medium (A, D); basal medium with seven vitamins, thiamine omitted (B, E); basal medium with seven vitamins, riboflavin omitted (C, F).

was substituted for asparagine. For these four fungi the dextrose concentration was reduced to 2 per cent and for *P. immitus* the solution was supplemented with a mixture of purine-pyrimidine bases. The reason for these modifications in the basal medium will be clarified later. The media were solidified with purified agar and observations on relative growth were made for a period of over 60 days.

TABLE 1. *Fungi showing complete deficiency for thiamine.*

Fomes fraxinophilus	59120S	*P. hydnans*	80938R
F. pini	52061	*P. immitus*	71384
F. roseus	10	*P. lucidus*	50390S
Polyporus abietinus	71429R	*P. obtusus*	71791S
P. anceps	58526R	*P. oregonensis*	1273
P. anceps	71825S	*P. osseus*	551
P. berkeleyi	53379S	*P. pargamenus*	57403S
P. borealis	91430	*P. pargamenus*	71954S
P. cinnabarinus	57071S	*P. pubescens*	71448R
P. croceus	47479S	*P. resinosus*	52043S
P. delectans	71089	*P. schweinitzii*	86353
P. delectans	71380S	*P. spumeus*	71929S
P. dichrous	56474S	*P. spumeus* v. *malicola*	18565S
P. distortus	57059S	*P. squamosus*	CBS-12A
P. durescens	71096	*P. tenuis*	57012
P. frondosus	72076S	*P. texanus*	94178R
P. fumosus	71335S	*P. trabeus*	55196R
P. giganteus	50382R	*P. tulipiferae*	71938
P. gilvus	56480S	*P. velutinus*	15067
P. graveolens	71021	*P. vulpinus*	2279
P. guttulatus	71799S		

Each of the 41 isolations (table 1) evidenced a complete deficiency for thiamine. No growth occurred in the tubes containing media which lacked thiamine even though the other seven vitamins were present; growth was obvious only on the media containing thiamine (fig. 2). Omission of any one of the other seven vitamins did not affect growth, except for *Polyporus texanus*, which failed to grow on a thiamine-free medium and on a medium lacking calcium pantothenate.

Response to thiamine intermediates. Previous investigations (Robbins and Kavanagh 1937, Müller and Schopfer 1937) have shown that the pyrimidine or thiazole intermediates may replace thiamine in the nutrient requirements of some fungi. To determine the response of the fungi included in this investigation each was grown in triplicate in test tubes on the basal agar medium and on the basal medium supplemented with thiamine, with thiazole, with pyrimidine, or with both intermediates at the rate of 10 mμ moles per tube. Observations on growth were made at intervals for a period of 120 days.

Each of the fungi tested grew on media supplemented with both intermediates; that is, none require thiamine as such (Robbins 1938, Robbins and

Ma 1943). None grew on media free from pyrimidine; that is, none were apparently able to synthesize the pyrimidine. Growth on the media supplemented with pyrimidine varied with the fungus. On the basis of the amount of growth on the media supplemented with pyrimidine the fungi were grouped in five groups (table 2).

TABLE 2. *Relative growth of various species of Hymenomycetes investigated on a basal medium and on the same medium supplemented with thiamine, thiazole, pyrimidine, or thiazole and pyrimidine. The species in each group A to E are given in the text.*

Fungus Group	Relative growth on basal medium supplemented with:				
	None	Thiamine	Thiazole	Pyrimidine	Thiazole + Pyrimidine
A	–	++++	–	–	++++
B	–	++++	–	+	++++
C	–	++++	–	++	++++
D	–	++++	–	+++	++++
E	–	++++	–	++++	++++

Group A included two fungi, *Polyporus graveolens* and *P. pargamenus* 71954S, which failed to grow on the pyrimidine medium (fig. 3).

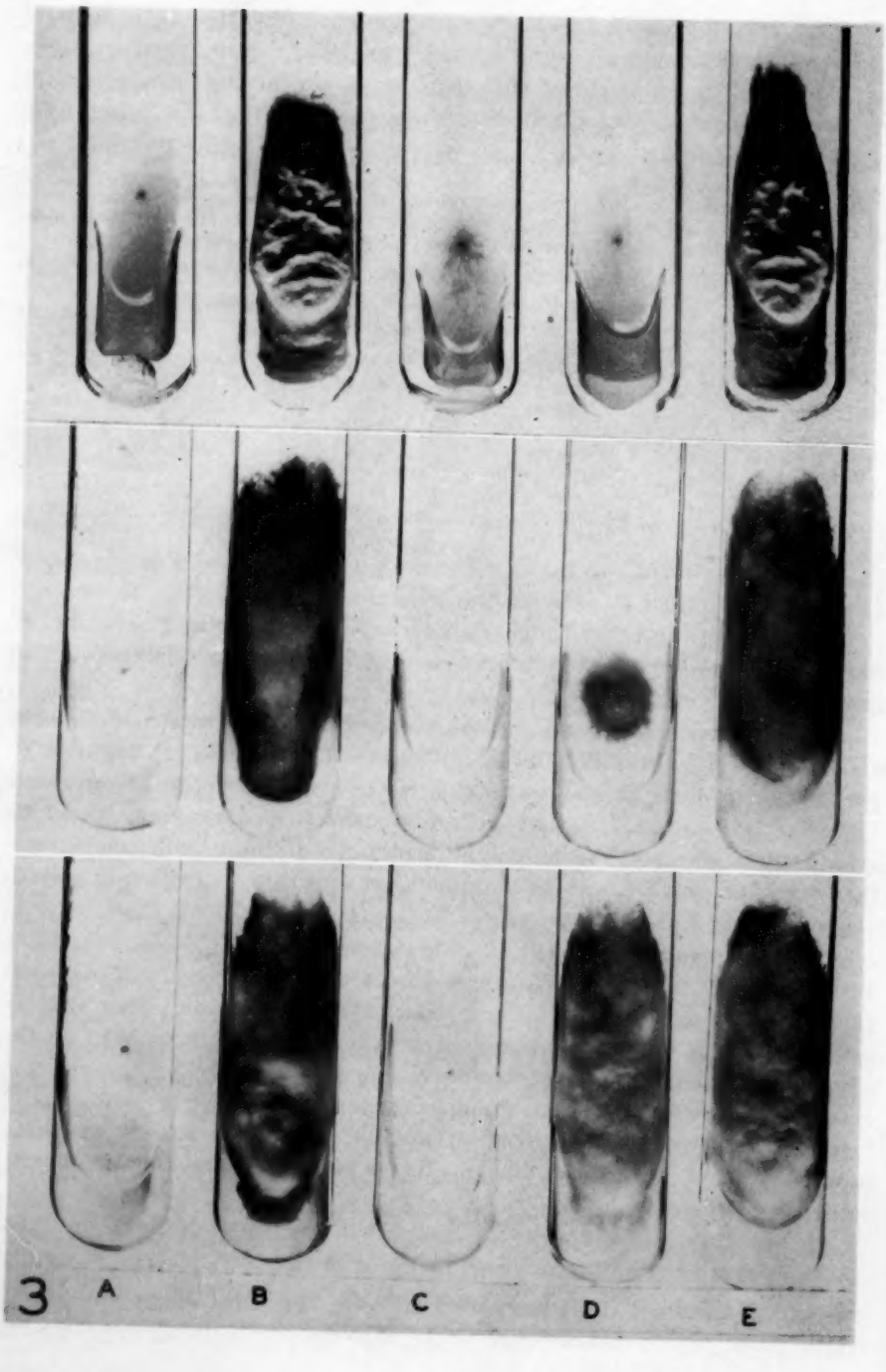
Group B included five fungi, *Polyporus anceps* 58526R, *P. croceus, P. durescens, P. hydnans* and *P. oregonensis,* which showed a small amount of growth on the thiazole-free medium.

Group C included *Fomes fraxinophilus, F. pini, F. roseus, Polyporus abietinus, P. delectans* 71380S, *P. guttulatus, P. immitus, P. lucidus, P. pargamenus* 57403S, *P. pubescens, P. resinosus, P. schweinitzii, P. spumeus* 71929S, *P. squamosus, P. texanus, P. trabeus* and *P. velutinus* (fig. 3), which grew at first markedly better on the medium containing both components than on that furnished with pyrimidine alone, but this distinction in growth disappeared in a short time.

Group D included *P. anceps* 71825S, *P. cinnabarinus, P. delectans* 71089, *P. dichrous, P. distortus, P. gilvus, P. osseus, P. spumeus* var. *malicola* 18565S, *P. tenuis* and *P. tulipiferae.* The fungi in this group grew slightly better at first on media supplemented with both intermediates than on the pyrimidine medium but the difference disappeared shortly.

Group E, including seven fungi, *Polyporus berkeleyi, P. borealis, P. frondosus, P. fumosus* (fig. 3), *P. giganteus, P. obtusus* and *P. vulpinus,* grew as well on the pyrimidine media as on the media furnished with both intermediates.

We interpret these results to mean that the fungi differed in their ability to synthesize thiazole. Those included in Group A lacked the ability completely. Those in Group E were able to make sufficient thiazole for maximum growth in the presence of pyrimidine under our conditions. Be-


3
A
B
C
D
E

tween the two extremes were organisms able to synthesize some thiazole but not enough for maximum growth; they were partially deficient for thiazole. It should be noted that differences in ability to synthesize thiazole existed for strains of the same species.

Fries (1938), Schopfer and Blumer (1940), Melin and Nyman (1941), Melin and Norkrans (1942), Noecker and Reed (1943), Rennerfelt (1944), Fries (1945) and Lindeberg (1946) found various Hymenomycetes to respond to pyrimidine or a mixture of the thiazole and pyrimidine components of thiamine. None was found which grew with thiazole alone or which required thiamine as such.

Polyporus distortus, thiamine and its components. During the investigation of the response of the fungi to thiamine and its intermediates it was noted that *P. distortus* produced a special type of spore-bearing fructification which was more prominent on media supplemented with pyrimidine than on those supplemented with both intermediates.

Thiazole supplied in thiamine or as such, appeared to interfere with the production of the fructifications. The most favorable amount of pyrimidine lay between 1 and 100 mμ moles per 10 ml. of medium. The fructifications were stunted also on thiamine media as compared with those on media containing equimolecular amounts of pyrimidine. Further investigation of the relation of thiamine and its components to this type of fructification of *P. distortus* is desirable.

Polyporus texanus and pantothenic acid. Two strains of *P. texanus*, 94178R and B734, were found to be completely deficient for thiamine and almost completely deficient for pantothenic acid.

The fungus was grown on the basal agar medium, the basal meduim plus thiamine, plus calcium pantothenate and plus thiamine and calcium pantothenate. Thiamine and calcium pantothenate were added at the rate of 10 mμ moles per tube. Media were solidified with purified agar and the cultures were run in triplicate. In addition to using the basal medium, the experiment was repeated with ammonium nitrate substituted for asparagine in an amount furnishing an equivalent quantity of nitrogen. Observations were made over a period of 75 days.

P. texanus 94178R did not grow on the basal medium with asparagine or ammonium nitrate as the source of nitrogen. Good growth occurred on media supplemented with both thiamine and calcium pantothenate. A small amount of growth developed on media supplemented with thiamine; the ammonium nitrate medium appeared slightly better than the medium con-

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FIG. 3. Response to thiamine intermediates. A, basal agar medium; B, basal medium plus 10 mμ moles of thiamine per tube; C, plus thiazole; D, plus pyrimidine; E, plus thiazole and pyrimidine. Above, *Polyporus pargamenus* 44 days old; middle, *P. velutinus*, 13 days old; below, *P. fumosus*, 13 days old.

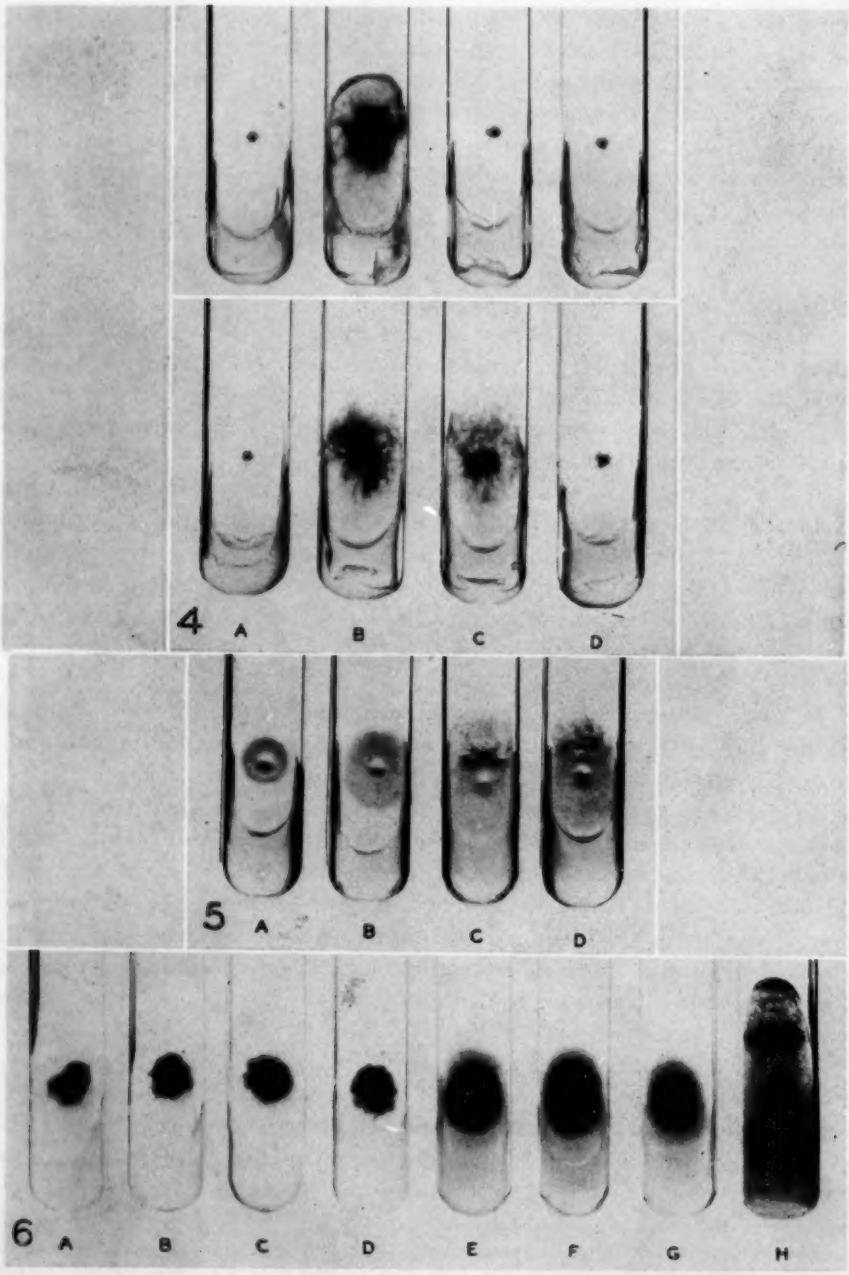

FIG. 4. Response of *Polyporus texanus* to pantothenic acid and its intermediates, age 28 days. A, basal agar medium; B, basal medium plus calcium pantothenate; C, plus butyrolactone; D, plus β-alanine. Above, 5 mμ moles of supplements per 10 ml. of

taining asparagine. No growth occurred on the medium supplemented with calcium pantothenate alone. Similar results were obtained with strain B734 so far as the effect of the vitamin supplements were concerned. For this strain, however, asparagine was more nearly equivalent to ammonium nitrate in its effect on growth on the media supplemented with thiamine.

Polyporus texanus and components of pantothenic acid. The effect of the two components of pantothenic acid. β-alanine and α-hydroxy-β, β-dimethylbutyrolactone, was investigated. The growth of the fungus on media containing equimolecular amounts of calcium pantothenate, β-alanine, the butyrolactone and mixtures of the two precursors, was compared on media solidified with purified agar (fig. 4) and in liquid media. The calcium pantothenate and its precursors were added to the basal medium supplemented with thiamine.

Two samples of butyrolactone (A and B) kindly supplied by Merck and Co. were tested. Sample (B) was specially purified and stated to be free of pantothenic acid and β-alanine. It was 90 to 95 per cent pure and may have contained some dl-pantoyl (butyro) lactone and cyanohydrin. The same results were obtained with both samples.

Calcium pantothenate and its lactone favorably affected the growth of *P. texanus* in a medium containing thiamine. However, substantially larger quantities of the lactone than of the calcium pantothenate were required to produce a given growth effect. Beneficial effects of calcium pantothenate were observed at 0.01 mμ moles per 11 ml. of medium. Butyrolactone gave a measurable effect when present at 50 mμ moles and the effect increased with 75 mμ moles. Larger amounts did not result in greater growth. β-alanine was ineffective and mixtures of the two precursors were of no greater benefit than equimolecular amounts of the lactone alone. These results were not caused by a contamination of the lactone with pantothenic acid.

Organisms requiring pantothenic acid for growth may not be able to synthesize it for one of a number of reasons. Some, lactic acid bacteria (Snell, Strong and Peterson 1939) and Pasteurella strains (Berkman 1942), are unable to carry out the coupling and require the intact pantothenic acid molecule. Other organisms, some yeasts (Williams, Eakin and Snell 1940, Lochhead and Landerkin 1942), and *Corynebacterium diphtheriae* (Mueller

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medium; below, 100 mμ moles per 10 ml. of medium. FIG. 5. Response of *Polyporus immitus* to adenine. Medium contains mineral salts, 1 per cent dextrose, casein hydrolysate and eight B vitamins. A, no addition; B, purine-pyrimidine bases without adenine; C, purine-pyrimidine bases without cytosine; D, all purine-pyrimidine bases. Age 12 days. FIG. 6. Response of *Polyporus schweinitzii* to oleic acid. A, basal agar medium; B, basal medium plus purine and pyrimidine bases; C, plus nucleic acid; D, plus choline; E, plus oleic acid; F, plus oleic acid and nucleic acid; G, plus oleic acid and choline; H, 2 per cent malt extract.

1937) require β-alanine, the butyrolactone moiety is apparently made by these organisms. Still other organisms, *Acetobacter suboxydans* (Underkofler, Bantz and Peterson 1943) and a hemolytic streptococcus (Wooley 1939) presumably synthesize β-alanine but fail to make the butyrolactone. The response of *P. texanus* does not appear to fit any of the types hitherto described.

Effect of malt extract. *Polyporus croceus, P. fumosus, P. giganteus, P. gilvus* and *P. pargamenus* produced more dry weight in 26 days in our basal liquid medium supplemented with vitamins and casein hydrolysate than in 2 per cent malt extract. On the other hand, *Fomes fraxinophilus, F. pini, F. roseus, Polyporus berkeleyi, P. borealis, P. frondosus, P. guttulatus, P. immitus, P. schweinitzii, P. texanus* and *P. vulpinus* produced substantially more dry matter in 2 per cent malt extract than on the basal medium supplemented with vitamins and casein hydrolysate. Each of the latter fungi was individually investigated to define more clearly why the 2 per cent malt extract was more satisfactory than the basal supplemented medium.

The basal solution used was that described earlier with 0.2 per cent asparagine and 5 per cent dextrose unless otherwise indicated. Casein hydrolysate was used at 0.12 per cent unless otherwise given. The vitamin mixture was that described earlier plus 30 mμ moles of folic acid (pteroylglutamic acid) per 25 ml. of medium. The malt extract contained 1.5 per cent nitrogen.

Malt "eluate" and malt "filtrate" were prepared by adding Norit A (in an amount equivalent to 0.1 to 0.25 of the weight of the malt extract) to a 2 per cent or 4 per cent malt extract and shaking for several hours. The solution was filtered and the filtrate retreated with Norit A. The filtrates were combined. The charcoal was washed with distilled water (washings added to the filtrate) and eluted with ammoniacal acetone (5 per cent ammonia and 60 per cent acetone) until the eluate showed no color. Both eluate and filtrate were concentrated on a hot plate. Each was used in an amount equivalent to 2 per cent malt extract. At this concentration about 40 mg. of solids were added as malt eluate to each culture (25 ml.) and 300 mg. as malt filtrate. On a dry weight basis the malt eluate contained 5 per cent nitrogen as compared to 1.5 per cent in the original malt extract. These determinations were made by Joseph F. Alicino.

A mixture (A) of purine and pyrimidine bases was used on occasion as a supplement. Mixture (A) was 500 mμ moles each of adenine, cytosine, guanine, hypoxanthine, thymine and uracil, 100 mμ moles of orotic acid and 10 mμ moles of xanthine per 25 ml. of medium.

A mixture (B) was also used as a supplement. Mixture (B) was 5 mg. each of dl-tryptophane and l-cystine, 0.03 ml. of oleic acid, 5 mg. of acetic acid, 0.5 mg. each of fumaric, succinic, malic and tartaric acids, 30 mμ

moles of hemin, 1000 mμ moles of choline chloride, 125,000 mμ moles of hydrolyzed nucleic acid and 125,000 mμ moles of ribose per 25 ml. of medium.

Fomes fraxinophilus. Almost no growth of *F. fraxinophilus* occurred in a basal solution containing mineral salts, asparagine and 5 per cent dextrose. The addition of vitamins to this medium had no beneficial effect but with a mixture of vitamins and casein hydrolysate 5.1 mg. of dry matter were obtained in 23 days (table 3). However, the yield in the same time in 2 per

TABLE 3. *Average dry weight in mg. of triplicate cultures in 25 ml. of a basal medium containing mineral salts, 5 per cent dextrose, 0.2 per cent asparagine and supplemented as indicated.*

Medium	*Fomes fraxinophilus* 23 d.	*F. pini* 27 d.	*F. roseus* 24 d.
Basal	0.2	0.6	1.2
Bas. plus vitamins	0.2	3.3	3.8
Bas. plus vit. and NH_4NO_3	0.2	4.1	3.7
Bas. plus vit. and cas. hyd.	5.1	12.7	7.0
Bas. plus vit. and eluate	3.3	10.4	10.0
Bas. plus vit. and filtrate	13.5	8.7	7.5
Bas. plus vit., eluate and filtrate	16.4	25.1	14.0
Bas. plus vit., cas. hyd. and eluate	11.1	33.3	20.5
2% malt extract	67.0	57.9	16.4
4% malt extract	105.6	105.4	33.7

cent malt extract was 67.0 mg. and in 4 per cent it was 105.6 mg. Similar results were obtained when the fungus was grown on the same media solidified with purified agar.

The basal medium described above was found to be unsatisfactory for a variety of reasons. It lacked thiamine for which this fungus, as was pointed out earlier, is completely deficient. The concentration of dextrose was too high; better growth was obtained with 1 or 2 per cent dextrose than with larger amounts. Asparagine was a poor source of nitrogen. Better growth was observed in the basal mineral medium containing vitamins and 2 per cent dextrose when ammonium sulfate was substituted for asparagine in an amount which gave an equal quantity of nitrogen. However, casein hydrolysate was markedly superior to ammonium sulfate. For example, on agar media containing mineral salts, vitamins and 2 per cent dextrose, no growth was visible after 21 days on the media where asparagine was furnished; the growth on the medium containing casein hydrolysate was estimated to be twice that on the medium containing ammonium sulfate.

However, even when the dextrose was reduced to 1 per cent, casein hydrolysate substituted for asparagine and vitamins added, growth was much less than in 2 per cent malt extract. For example, in 25 days no growth occurred in the basal medium with asparagine and 5 per cent dextrose; the

addition of vitamins and 0.12 per cent casein hydrolysate gave 16.4 mg.; reducing the dextrose to 1 per cent and substituting casein hydrolysate for asparagine in the vitamin-supplemented medium produced 26.3 mg., but in 2 per cent malt 81.1 mg. were obtained.

Malt extract apparently furnished some growth supplement not included in the mineral salts, casein hydrolysate, vitamins or dextrose of our meduim. This assumption is supported by the effect of small amounts of malt extract. For example, the addition of 0.05 per cent malt extract (12.5 mg. per flask) to a medium containing mineral salts, 1 per cent dextrose, vitamins and 0.24 per cent casein hydrolysate increased the growth from 25.7 mg. 51.2 mg. (table 4). This cannot be accounted for on the basis of the addition of nitro-

TABLE 4. *Average dry weight in mg. of triplicate cultures of fungi grown in 25 ml. of the media indicated. Three basal media were used. In addition to mineral salts basal medium I contained 5% dextrose and 0.2% asparagine; II, 1% dextrose and 0.12% casein hydrolysate; III, 1% dextrose and 0.24% casein hydrolysate.*

Medium	*Fomes fraxinophilus Bas. med. III* 25 d.	*Polyporus frondosus Bas. med. I* 21 d.	*P. schweinitzii Bas. med. I* 26 d.	*P. schweinitzii Bas. med. III* 23 d.	*P. immitus Bas. med. II* 23 d.
Basal	0.3	2.7	1.2	0.5	.....
Bas. plus vitamins	.....	6.6	4.7	.....	.....
Bas. plus vit. and cas. hyd.	25.7	10.5	12.6	5.4	6.3
Bas. plus vit., cas. hyd. and following amts. Malt ext.					
0.05%	51.2	.....	.....	43.8	10.2
0.1	51.8	18.2	29.9	53.9	12.8
0.5	.....	25.8	34.5	.....	.....
1.0	85.5	25.9	45.6	98.6	.....
2.0	111.4	29.1	30.9	72.7	.....
4.0	93.6	34.6	30.1	52.2	.....
1% malt extract	53.4	.....	.....	42.8	.....
2% malt extract	81.1	.....	.....	82.4	14.1
4% malt extract	93.5	.....	.....	95.3	.....

gen. The addition of 0.05 per cent malt extract would represent 0.625 mg. of nitrogen per flask. However, 0.24 per cent casein hydrolystate supplied more than an adequate amount of nitrogen because reducing the casein hydrolysate to 0.12 per cent did not affect growth.

The beneficial material in malt extract was, at least in part, adsorbed on Norit A from which it could be eluated. The eluate duplicated in part the action of casein hydrolysate but was effective also in the presence of casein hydrolysate.

We were not able to identify the factor present in malt extract which favorably affected the growth of *F. fraxinophilus*. The addition of mixture (A) to an agar medium containing 2 per cent dextrose, vitamins and 0.12 per cent casein hydrolysate was of no benefit. The further addition of mix-

ture (B) approximately doubled growth in 16 days but the growth was about two-thirds of that on 2 per cent malt extract agar. Efforts to identify the substances in the above mixture responsible for the beneficial effect were not entirely successful. The oleic acid, the ribose and the hydrolyzed nucleic acid seemed to be of more importance than the other constituents. However, growth on an agar medium containing mineral salts, 2 per cent dextrose, 0.12 per cent casein hydrolysate and vitamins and supplemented with oleic acid, ribose and hydrolyzed nucleic acid, was markedly inferior to that obtained on 2 per cent malt extract.

Fomes pini and F. roseus. Little growth of these two species was observed in the basal medium. The addition of our vitamin mixture improved the growth which was increased by the further addition of casein hydrolysate (table 3). However, growth of *F. pini* in 2 per cent malt extract was more than four times that in the basal medium supplemented with vitamins and casein hydrolysate and the growth of *F. roseus* in 2 per cent malt extract was twice that in the supplemented basal medium. NH_4NO_3 was not a satisfactory substitute for casein hydrolysate for either organism.

The malt eluate had much the same effect on growth as casein hydrolysate; the malt filtrate was somewhat less effective. This suggests that the eluate was active, at least in part, because of its amino acid content. A further study of these two species involving their relation to amino acids seems to be indicated. Both show a deficiency for vitamins (thiamine) and perhaps partial deficiencies for one or more amino acids.

Polyporus berkeleyi. The growth of *P. berkeleyi* in the basal solution containing 5 per cent dextrose and 0.2 per cent asparagine was negligible. Growth was slightly improved by the addition of vitamins and a further slight improvement was obtained with casein hydrolysate (table 5). However, growth was still about one-seventh of that in 2 per cent malt extract. The substitution of NH_4NO_3 for casein hydrolysate was ineffective; malt eluate was more effective than casein hydrolysate. The action of the eluate and that of casein hydrolysate in the amounts used were roughly additive.

Asparagine was found to be inferior to either ammonium salts or casein hydrolysate as a nitrogen source although the differences in growth tended to disappear in older cultures.

The effect of the addition of mixture (A) to a medium containing vitamins and casein hydrolysate was inconclusive. In some experiments a slight benefit was observed; in others there was none. Sodium oleate appeared to be of some importance but greater benefits were observed in agar media with sodium arachadonate. Liver extract added at the rate of 23.6 mg. per cent to the basal agar medium containing 2 per cent dextrose, vitamins, purine and pyrimidine bases and casein hydrolysate stimulated growth but it was

less than on 2 per cent malt extract agar. Vitamin B_{12} added to the same medium was ineffective.

While it is clear that *P. berkeleyi* suffers from a complete deficiency for thiamine and perhaps a partial deficiency for one or more amino acids, the effectiveness of malt extract, especially of the eluate, is not completely defined.

TABLE 5. *Average dry weight in mg. of triplicate cultures grown in 25 ml. of the media indicated. Basal medium contained mineral salts, 5 per cent dextrose and 0.2 per cent asparagine.*

Medium	*Polyporus berkeleyi* 21 d.	*P. frondosus* 28 d.	*P. immitus* 35 d.	*P. tulipiferae* 14 d.
Basal	0.7	1.3	0.2	0.4
Bas. plus vitamins	3.1	14.5	0.8	17.6
Bas. plus vit. and NH_4NO_3	3.0	12.4	0.9	44.2
Bas. plus vit. and cas. hyd.	5.1	25.2	4.1	64.1
Bas. plus vit. and eluate	18.8	71.4	2.3	77.2
Bas. plus vit. and filtrate	17.7	42.9	13.0	68.2
Bas. plus vit., eluate and filtrate	30.9	111.3	17.8	105.8
Bas. plus vit., cas. hyd. and eluate	22.7	94.3	3.1	110.4
2% malt extract	35.0	77.1	29.6	51.8
4% malt extract	51.1	114.5	26.9	79.8

Polyporus frondosus. The results for this fungus were qualitatively much like those obtained with *P. berkeleyi;* the amount of growth was greater.

P. frondosus evidenced vitamin heterotrophy (for thiamine) and perhaps a partial defiicency for one or more amino acids as indicated by the beneficial effects of casein hydrolysate (table 5). Malt eluate not only replaced casein hydrolysate but was effective in the presence of casein hydrolysate. This suggests that malt extract contains unidentified substances needed in the nutrition of this fungus. This conclusion is supported by the observation that the addition of 0.1 per cent malt extract (circa 3.8 mg. of nitrogen per flask) to the basal medium containing vitamins and casein hydrolysate nearly doubled the growth (table 4).

Polyporus immitus. Almost no growth of *P. immitus* occurred in a basal medium containing mineral salts, 5 per cent dextrose and asparagine. The addition of a mixture of B vitamins had little effect. With the further addition of casein hydrolysate appreciable growth amounting to 4.1 mg. was obtained. However, in 2 per cent malt extract the yield was 29.6 mg. (table

4). The response of *P. immitus* to malt extract could not be completely accounted for by the vitamins included in our B mixture and the constituents of casein hydrolysate.

A marked improvement in growth was obtained when mixture (A) was added to the basal medium containing 1 per cent dextrose and no asparagine but supplemented with B vitamins and casein hydrolysate. The dry weight in the basal medium without the purine and pyrimidine bases was 6.8 mg.; with the purine and pyrimidine bases it was 12.9 mg. In the same experiment 8.5 mg. dry weight were obtained with 1 per cent malt extract.

The effective substance was found to be adenine (fig. 5). When adenine sulphate was added to a modified basal solution containing 1 per cent dextrose and no asparagine but supplemented with a mixture of B vitamins and casein hydrolysate, a beneficial effect was noted with 0.04 mμ mole of adenine per ml.; maximum effects were secured with between 4 and 20 mμ moles per ml.

The beneficial effect of malt extract as compared to a basal medium containing mineral salts, dextrose and asparagine, may be largely accounted for by the amino acids, thiamine and adenine present in the malt extract. A "synthetic" medium having the following composition supported as much growth of *P. immitus* as 2 per cent malt extract: KH_2PO_4 1.5 gm., $MgSO_4 \cdot 7H_2O$ 0.5 gm., dextrose 10–30 gm., mixture of micro essential elements 0.5 ml., casein hydrolysate 1.2 gm., thiamine hydrochloride 1000 mμ moles, adenine sulphate 20,000 mμ moles, distilled water to make 1 liter.

Polyporus schweinitzii. Two strains of this fungus were investigated, 94095R and 86353 respectively. Both grew very poorly on the basal meduim. The growth of the first strain was improved by the addition of vitamins and casein hydrolysate. However, the dry weight obtained in 2 per cent malt extract was nearly twice that in the supplemented basal medium. The second strain, 86353, differed from the first in showing less response to the vitamins or to the mixture of vitamins and casein hydrolysate. The experiments summarized below were performed with this strain.

Casein hydrolysate was found to be a better source of nitrogen than ammonium sulfate or asparagine. Better growth was obtained in the basal medium, supplemented with vitamins and casein hydrolysate, with 1 or 2 per cent dextrose than with 3, 4 or 5 per cent. However, reducing the dextrose to 1 per cent and substituting casein hydrolysate for asparagine in the basal solution supplemented with vitamins did not produce a medium as satisfactory as 2 per cent malt extract. For example, in 18 days the growth in 2 per cent malt extract was 53.2 mg. and in the basal solution containing 1 per cent dextrose, 0.12 per cent casein hydrolysate and vitamins, it was 2.7 mg.

The presence of unidentified favorable factors in malt extract is suggested by the effect of the addition of small amounts of malt extract to the basal medium containing vitamins and casein hydrolysate. For example, the addition of 0.05 per cent malt extract (12.5 mg. per flask) to the basal medium containing 1 per cent dextrose, vitamins and 0.24 per cent casein hydrolysate increased growth from 5.4 mg. to 43.8 mg. (table 4).

We did not succeed in identifying the favorable factors in malt extract. They were not concentrated in an acetone extract of malt; there was some evidence that the unidentified factors were adsorbed in part on Norit A.

The addition of mixture (A) to the basal agar medium containing 2 per cent dextrose, vitamins and 0.12 per cent casein hydrolysate was ineffective. The further addition of mixture (B) resulted in some improvement in growth. Oleic acid appeared to be the effective material in this mixture (fig. 6). Some benefit was observed with 0.025 mg. per ml.; the optimum amount was 0.25 mg. per ml. This effect was not produced by Tween 80 nor by methyl oleate, sodium linolenate, methyl linolenate or sodium linolate. The results with arachadonic acid were inconclusive.

However, our best medium containing vitamins, casein hydrolysate and oleic acid was much less satisfactory than 2 per cent malt extract. Further efforts to clarify the nutrient requirements of *P. schweinitzii* are desirable.

Polyporus tulipiferae. The growth of this fungus on our basal medium was insignificant. The addition of vitamins appreciably improved growth which was further increased by the addition of casein hydrolysate (table 5). Growth in the basal medium supplemented with vitamins and 0.12 per cent casein hydrolysate surpassed that in 2 per cent malt extract. Asparagine was a poor source of nitrogen; both NH_4NO_3 and casein hydrolysate were better. Reducing the dextrose from 5 per cent to 1 per cent improved growth. It seems probable that our basal medium with NH_4NO_3 instead of asparagine, 1 per cent dextrose and vitamins (thiamine) would allow as great or greater growth than 2 per cent malt extract.

Polyporus borealis, P. guttulatus, P. texanus and P. vulpinus. None of these species grew on the basal medium containing 5 per cent dextrose and 0.2 per cent asparagine. The addition of the vitamin mixture substantially improved the growth of *P. borealis, P. texanus* and *P. vulpinus* but had little or no effect on *P. guttulatus* (table 6). However, growth of *P. borealis, P. texanus* and *P. vulpinus* in the basal medium supplemented with vitamins was half (or less) that obtained in 2 per cent malt extract. The greater growth of these three species in the malt extract as compared to that in the basal medium can only be partially explained by the presence in the malt extract of thiamine.

The addition of NH_4NO_3 to the medium containing vitamins was of no benefit to any of the four species. The addition of casein hydrolysate did not

increase the growth of *P. borealis* but had a marked beneficial effect on the growth of the other three species.

While no dogmatic statement can be made, it seems probable that one or more of the constituents of the basal medium (probably dextrose) may be present in too great a concentration for the most satisfactory growth of *P. borealis*. This is suggested by the failure to obtain growth equal to that in 2 per cent malt extract on the addition of the malt eluate and filtrate to the basal medium supplemented with vitamins and casein hydrolysate (table 6). The lack of growth of *P. borealis* on our basal medium is evidently due primarily to a lack of vitamins (thiamine). The more satisfactory results with 2 per cent malt extract may be associated with the greater dilution of this medium as compared to our basal medium containing vitamins.

TABLE 6. *Average dry weight in mg. of triplicate cultures in 25 ml. of a basal medium containing mineral salts, 5 per cent dextrose and 0.2 per cent asparagine and supplemented as indicated.*

Medium	*Polyporus borealis* 27 d.	*P. guttulatus* 37 d.	*P. texanus* 27 d.	*P. vulpinus* 28 d.
Basal	0.1	0.2	0.5	0.8
Bas. plus vitamins	23.7	0.4	12.1	13.1
Bas. plus vit. and NH_4NO_3	25.2	0.3	8.7	10.1
Bas. plus vit. and cas. hyd.	19.8	13.8	45.4	40.9
Bas. plus vit. and eluate	25.7	0.2	33.4	30.5
Bas. plus vit. and filtrate	11.7	6.6	51.8	46.5
Bas. plus vit., eluate and filtrate	24.6	14.9	63.0	80.2
Bas. plus vit., cas. hyd. and eluate	17.3	15.3	60.8	86.3
Bas. plus vit., cas. hyd., eluate and filtrate	22.4	25.3	40.0	117.2
2 per cent malt ext.	45.0	29.3	51.9	47.7
4 per cent malt ext.	52.6	44.9	39.8	81.7

Whatever was supplied *P. guttulatus* by the casein hydrolysate was not furnished by malt eluate but was present to some degree in the malt filtrate. Neither asparagine nor NH_4NO_3 was utilized by this fungus under our conditions; casein hydrolysate was a good source of nitrogen. However, growth in the basal medium supplemented with both vitamins and casein hydrolysate was about one half that obtained in 2 per cent malt extract (table 6). It appears justifiable to conclude that *P. guttulatus* suffers from vitamin deficiencies (thiamine) and requires organic nitrogen. Further investigation is needed to define the effect of malt extract.

P. vulpinus grew more in 4 per cent malt extract than in 2 per cent malt extract; the opposite was true for *P. texanus*. Otherwise, the response of these two species was much the same and they are discussed together. Each grew approximately as well in the basal medium supplemented with vitamins and casein hydrolysate as they did in 2 per cent malt extract. However, malt eluate had an effect on these two fungi which requires further investigation. In contrast to its action on *P. guttulatus* malt eluate took the place in part of casein hydrolysate, as may be noted by comparing the growth in the basal vitamin medium with that in the same medium supplemented with casein hydrolysate or with malt eluate (table 6). Furthermore, the malt eluate markedly improved growth in the basal medium supplemented with both vitamins and casein hydrolysate. Whether the action of the malt eluate was qualitative or quantitative cannot be determined from these data. *P. texanus* suffers from a deficiency for vitamins (thiamine) and pantothenic acid and a partial deficiency for one or more of the constituents (probably amino acids) of casein hydrolysate; *P. vulpinus* is heterotrophic for thiamine and one or more of the constituents of casein hydrolysate.

Discussion. Previous investigations have shown that many species of Hymenomycetes are heterotrophic for thiamine. Over 100, including species of *Amanita, Aricularia, Boletus, Cenococcum, Clavaria, Clitocybe, Clitopilus, Collybia, Coprinus, Cortinellus, Dachryomyces, Daedalea, Flammula, Fomes, Hydnum, Hypholoma, Lactarius, Lentinus, Lenzites, Lepiota, Marasmius, Merulius, Mycena, Panus, Pholiota, Polyporus, Poria, Psalliota, Ptychogaster, Rhizopogon, Schizophyllum, Stereum, Stropharia, Trametes, Tricholoma* and *Tubularia*, have been found to be completely or partially heterotrophic for thiamine. For some species the pyrimidine moiety of thiamine, or a mixture of pyrimidine and thiazole, substitutes for thiamine. None of the Hymenomycetes are known to require the intact thiamine molecule. Melin and Norkrans (1942) state that thiazole partially replaces thiamine for *Boletus luteus* and *B. piperatus*. Our investigations of *Polyporus* and *Fomes* have added a number of species to those which are heterotrophic for thiamine. Ability to synthesize the constituents of the vitamin was found to vary with the species, and in some instances, with the strain.

Some of the Hymenomycetes have been found to be heterotrophic for biotin (Melin and Lindeberg 1939, Melin and Nyman 1940 and 1941, Lindeberg 1941, 1944 and 1946, and Treschow 1944). None of our species evidenced a biotin requirement.

Jennison and Henderson (1949) state that a number of species of wood destroying fungi were markedly stimulated by the addition of adenine when NH_4SO_4 was the source of nitrogen and that the effect of purine and pyrimidine bases was especially noteworthy when inorganic nitrogen was used. We found the effect of the purine and pyrimidine bases under our conditions to

be minor except for *Polyporus immitus,* which was heterotrophic for thiamine and for adenine. *P. texanus* is especially noteworthy because of its need for pantothenic acid, a requirement which has not been hitherto reported for any of the Hymenomycetes.

The relation of the Hymenomycetes to nitrogen requires further investigation. Melin and Mikola (1948) found amino acids to have a stimulating effect on *Cenococcum graniforme.* Melin and Norkrans (1948) observed that casein hydrolysate benefited growth of *Lactarius deliciosus* in a mineral solution containing dextrose, ammonium tartrate and thiamine. A mixture of 18 amino acids as present in the casein was beneficial also. Jennison and Henderson (1949) found for their organisms organic nitrogen (glutamic acid) to be superior to inorganic nitrogen and state that almost any amino acid could be substituted for glutamic acid.

Our investigations emphasize that species vary in their relation to nitrogen. For example, asparagine was a poor source of nitrogen for *Fomes fraxinophilus, Polyporus berkeleyi, P. schweinitzii* and *P. tulipiferae;* ammonium salts were better, but casein hydrolysate was the best. There were differences within this group; little or no growth of *Fomes fraxinophilus* occurred on asparagine media while *Polyporus tulipiferae* did fairly well. *P. guttulatus* and *P. immitus* grew very little with asparagine or ammonium salts; these fungi grew well with casein hydrolysate as the nitrogen source. The growth of *P. frondosus* was best on asparagine or casein hydrolysate media; ammonium salts were inferior. In general, casein hydrolysate was a better source of nitrogen for the fungi we dealt with than either asparagine or ammonium salts.

Oleic acid improved the growth of *Polyporus berkeleyi, P. frondosus* and *P. schweinitzii.* Sodium oleate was active at 0.025 mg. per ml., but for maximum effects 0.1 to 0.5 mg. per ml. was required. Benham (1941) found fats or fatty acids indispensable for *Pityrosporum ovale.* Bauernfeind, Sotier and Boruff (1942) report that stearic, palmitic or linoleic acid stimulated *Lactobacillus casei* in the presence of suboptimum amounts of riboflavin. Kodicek and Worden (1944) found palmitic and stearic acid to augment growth of *Lactobacillus helveticus.* Hutchings and Boggiano (1947) reported sodium oleate to benefit species of lactobacilli.

However, the importance of oleic acid as a constituent of malt extract is dubious. This is suggested by the observation that the addition of 1 mg. of malt extract per ml. to the basal medium produced in some instances a substantial increase in growth. To account for this effect on the basis of oleic acid would necessitate assuming 9 per cent oleic acid in malt extract, an assumption which is obviously invalid.

Does malt extract contain unidentified substances important for the

growth of some species of *Polyporus* and *Fomes?* We interpret our results to suggest this possibility.

The better growth of a fungus on a natural medium, for example malt extract, as compared to that in a medium of sugar, mineral salts and a source of nitrogen may be the result of the action of one or more of several factors. The hydrion concentration of the basal medium may be unfavorable; its buffer action may be too great or too little; its osmotic concentration may be ill-adjusted; or some constituent of the medium may be toxic. On the other hand, the basal medium may not supply essential mineral elements, essential metabolites or suitable sources of carbon or of nitrogen in adequate quantity.

We do not believe that hydrion concentration and buffer action were major factors in our experiments. The effect of variations of initial pH from 3.75 to 5.85 on the growth of *Fomes fraxinophilus, Polyporus frondosus, P. immitus* and *P. schweinitzii* in our basal medium supplemented with vitamins and casein hydrolysate was insufficient to explain the greater growth of these fungi in malt extract. A comparison of pH values before and after growth in the various media led to the same conclusion. The buffer action of 2 per cent malt extract and that of our basal medium supplemented with vitamins and casein hydrolysate was much the same. The addition of malt ash to the basal medium containing vitamins and casein hydrolysate had little effect on the growth of *P. frondosus, P. immitus* or *P. schweinitzii,* and did not account for the malt effect.

Reducing the dextrose concentration from 5 per cent to 1 or 2 per cent and substituting casein hydrolysate for asparagine improved the growth of some of the fungi investigated. However, for several, especially *Fomes fraxinophilus, Polyporus berkeleyi, P. frondosus* and *P. schweinitzii,* the superior growth on malt extract could not be accounted for by reducing the concentration of dextrose or by adding the constituents of casein hydrolysate, known vitamins, purine and pyrimidine bases, oleic acid or other supplements. Unidentified factors in malt extract appeared to play a role in the nutrition of these fungi. This assumption was supported by the marked effects of small amounts of malt extract or of a malt eluate when added to a medium containing mineral salts, dextrose, vitamins and casein hydrolysate. The beneficial action of the malt extract or of the malt eluate is not considered to be the result of an increased supply of nitrogen; it may be associated with specific compounds of nitrogen or other types of essential metabolites.

SUMMARY

1. Each of 41 species or strains of *Polyporus* and *Fomes* selected because of their poor growth on a basal medium of mineral salts, dextrose and asparagine was found to be heterotrophic for thiamine. None required thiamine as such. Thiamine could be replaced by pyrimidine or by a mixture

of the thiazole and pyrimidine components of thiamine. *Polyporus texanus* was found to be heterotrophic for both thiamine and pantothenic acid. Pantothenic acid could be replaced by butyrolactone but larger amounts were required; β-alanine was ineffective alone or with the lactone. *Polyporus immitus* was heterotrophic for thiamine and adenine.

2. About half of the species investigated grew better on 2 per cent malt extract than on the basal medium containing mineral salts, 5 per cent dextrose, asparagine, casein hydrolysate and B vitamins. The growth of some species was improved by reducing the dextrose to 1 or 2 per cent and substituting casein hydrolysate for asparagine. The "malt effect" for *Polyporus immitus* could be accounted for by the adenine supplied by the malt extract. The growth of some of the species (*P. schweinitzii* for example), was improved by the addition of oleic acid. However, malt extract appeared to furnish some of the species growth supplements not included in mineral salts, dextrose, casein hydrolysate, eight B vitamins, purine and pyrimidine bases, tryptophane, cystine, hemin, choline, hydrolyzed nucleic acid, ribose or oleic acid. The "malt factor" for these species was not identified.

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STUDIES ON ANTHOCEROTALES. IV

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It has often been pointed out that the Anthocerotales are in need of critical revision. On the specific level it seems reasonable to commence with a re-examination of those species which were described during the first century after Linné. It is understandable that the early authors, with a more limited need for comparison, wrote descriptions which often are more at the generic and even family level. Illustrations are often lacking entirely. As a result, many of the old species are for all purposes quite undefined in the modern sense; only a study of their types will make their circumscription possible. In the absence of such re-evaluation many of these taxa have often been redescribed as new. A study of the Linnean species was presented in the first sections of this series of papers (Proskauer 1948a). Now it has been possible to examine the type specimens of the species created by Montagne. For this opportunity I am most grateful to the authorities of the Muséum National d'Histoire Naturelle, Paris, and in particular to Madame S. Jovet-Ast who took much trouble on my behalf.

It must be emphasized that examination of herbarium material of this group has marked limitations. In general, the sporophyte, especially in its indurated cells, yields relatively more tangible features than the gametophyte. The shape of the gametophyte can be observed, but as these plants are highly plastic (Proskauer 1948a) one must remember that a particular collection usually will present but one of several possible phenotypic expressions. Older collections tend to lack data on microclimatic conditions. The whole possible range of form can only be evaluated from a study of living material. Exsiccatae rarely allow for satisfactory observation of anatomical features. Sex-distribution can only be decided positively if the species is monoecious and the specimens happened to bear both male and female organs at the time of collection. The chloroplasts tend to fragment during exsiccation, and observations on this important feature are so unreliable that they best are omitted. Chromosome morphology, now known to be of considerable taxonomic importance in the group (Proskauer 1951), cannot be studied in ordinary herbarium material with current methods. Such morphological information as can be gleaned may be of some profit nevertheless, as the following sections may indicate.

5. **Anthoceros dissectus** Mont. = Podostemaceae sp. This Brasilian plant was described by Montagne in 1838. The material was collected by Auguste de Saint-Hilaire. The packet bears the additional annotation "Nees n. 229".

It grew on rocks in the water of the river Hyta (*sic!*), in the general area of Rio de Janeiro (Montagne 1838 and Gottsche et al. 1844–47, differ as to the distance from that town). Montagne appears to have been somewhat puzzled by the material. Its habitat reminded him of the leaf of *Ranunculus aquatilis*. Figure 1 shows the single specimen in the packet. It appears to be

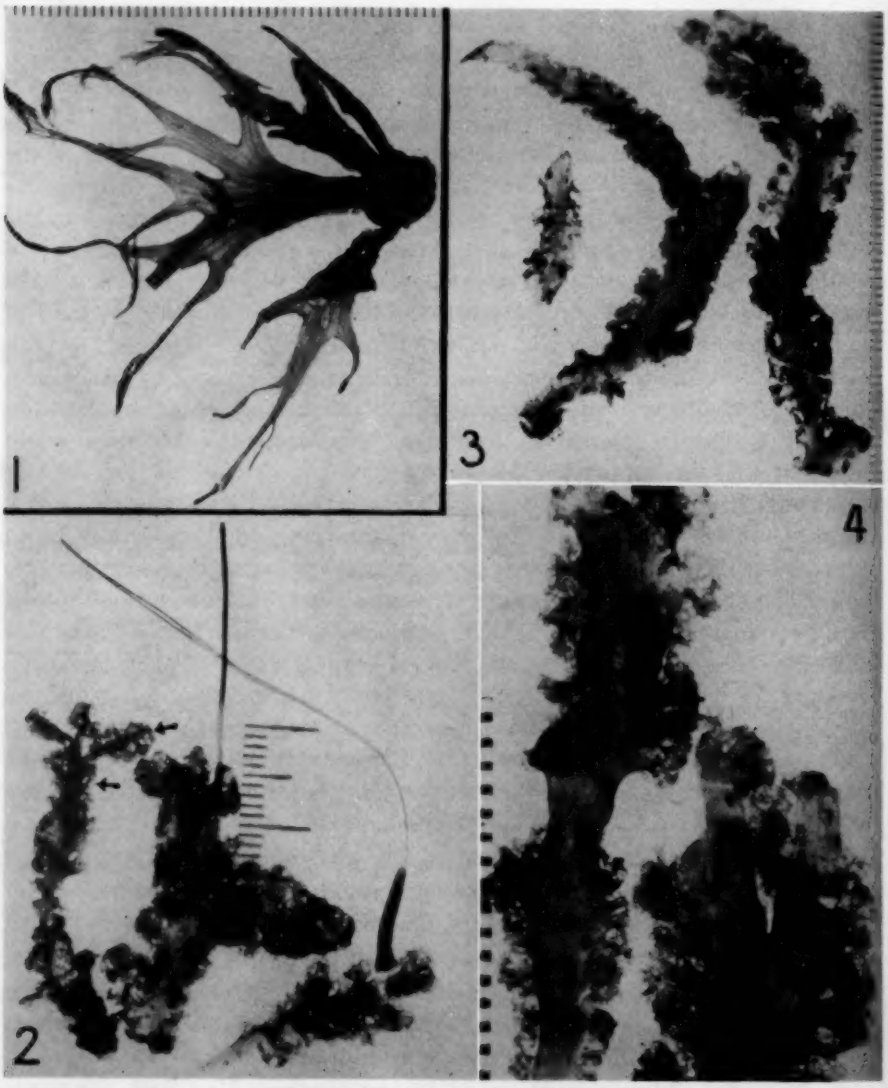


FIG. 1. *'Anthoceros' dissectus* (Podostemaceae). Type specimen. FIG. 2. *Dendroceros endiviaefolius*. Fertile material collected by Dusén. Arrows mark groups of antheridial cavities. FIGS. 3 & 4. Some of the type material. All scale intervals 1 mm.

the sterile portion of a member of the Podostemaceae. Gottsche et al. (1844–47) thought it might be part of a *Mourera*, and Montagne later (1856) stated that it appeared to belong to *Lacis*. The species may possibly be valid in the Podostemaceae.[1]

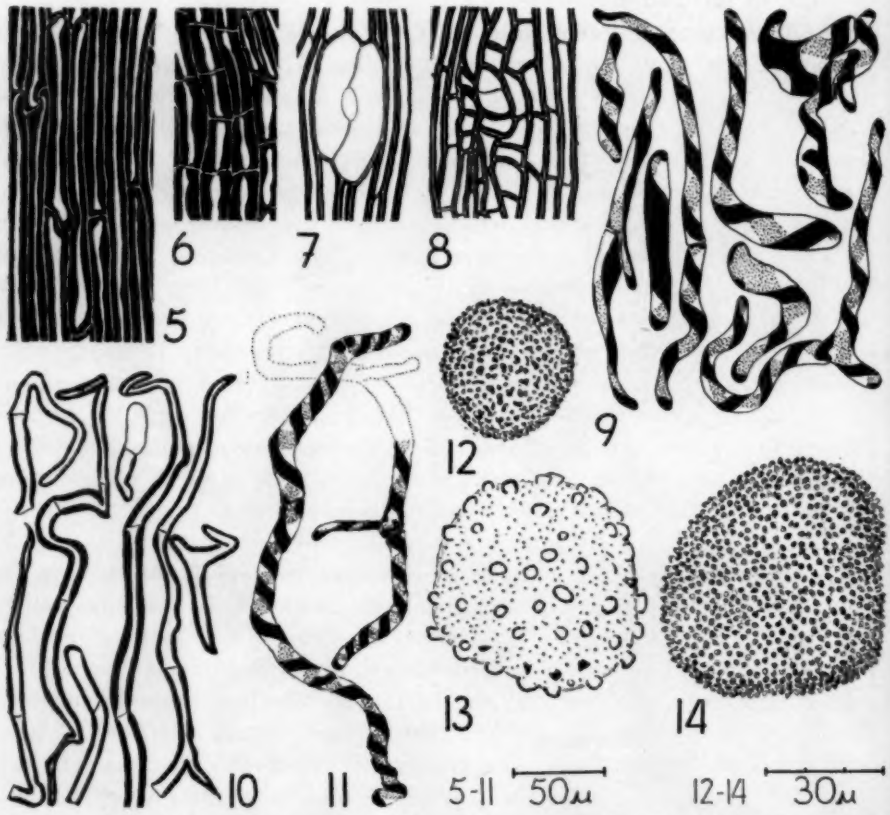
6. **Anthoceros endiviaefolius** Mont. = *Dendroceros endiviaefolius* (Mont.) comb. nov. The material was collected by Jacquinot at Puerto Hambre, 55 km south of Punta Arenas, Prov. Magallanes, Chile. Montagne (1845b, 1850), probably mindful of the earlier case of his *Anthoceros dissectus*, was not sure whether this sterile plant belonged to the Anthocerotaceae or Podostemaceae; he placed it in *Anthoceros*, but with a question mark. In 1856 he finally stated definitely that it appeared to belong to *Lacis*. Part of the type material is shown in figure 3. The plants are reminiscent of male thalli of *Riccardia pinguis* and Gottsche referred them there, according to his annotation label.[2] Only close examination (fig. 4) reveals that his view is erroneous. The strap-shaped thalli bear irregularly laciniate much folded wings, which are mainly unistratose. Colonies of *Nostoc* are present in the midrib, a fact verified by dissection. This, together with the meager information which can be gleaned about the cellular organization, places the plant in the Anthocerotales. The only additional piece of information, not shown in the photographs, was provided by a section through the thallus. In this section the midrib was some twenty cells high.

Additional and fertile material of this organism was kindly lent to me from the University Herbarium, København, Denmark. It was collected by P. Dusén at Puerto Angosto, Isla Desolación, Prov. Magallanes, on the first of April, 1896 (Stephani 1901). This material provided some further information concerning the anatomy of the thallus. Sections showed relatively small cavities, not much larger than the ordinary central midrib cells, filled with mucilage. Similar cavities occur in species of *Megaceros*. It is not clear if the cavities here represent intracellular spaces, as in *Anthoceros*, or idioblasts. The walls of the central cells of the midrib show a coarse reticulation, due to the presence of large pits. This pitting appears to have been overlooked previously in the Anthocerotales, but is a characteristic feature of *Megaceros* and certain species of *Phaeoceros*. Three of the fertile specimens are shown in figure 2. Antheridial cavities (see areas marked by arrows in thallus on left side of figure) were only found on lobes not connected with any bearing sporophytes. It is possible that this species is dioecious. The antheridia are found singly in the cavities, their walls are of the "phaeoceroid" type (Proskauer 1951). The longest of the four sporophytes present

[1] Since this was written Dr. P. van Royen, of Leiden, has informed me that *Apinagia dissecta* (Wedell) Engler appears to be based on the Montagne material. He will discuss the matter further in a forthcoming paper on the Podostemaceae of the New World.

[2] Delendus! Planta, quae ita a Doctorissimo Montagneo nuncupata est, est Aneura pinguis ♂. Gottsche.

on the material measured 9.5 cm. The valves remain connected apically after dehiscence, when dry they show the normal spiral twisting. Their epidermis lacks stomata (fig. 5). The lining layer (Proskauer 1948b) is very well de-



FIGS. 5, 9 and 13. *Dendroceros endiviaefolius* (material leg. Dusén). FIG. 5. Part of epidermis of sporophyte. FIG. 9. Variation in structure of elaters. FIG. 13. Outer face of spore; the drawing is accurate. FIGS. 6, 11 and 14. *Dendroceros cichoraceus* (type material). FIG. 6. Part of epidermis of sporophyte. FIG. 11. One unbranched and one branched (less common) elater. FIG. 14. Spore; the drawing is partially accurate. FIGS. 7, 10 and 12. *Anthoceros fuciformis* (type material). FIG. 7. Part of epidermis of sporophyte, with stoma. FIG. 10. Entire short and medium elaters, and fragments of long elaters. FIG. 12. Outer face of spore, the drawing is largely accurate. FIG. 8. *Anthoceros* sp. (Cameroons). Part of epidermis of sporophyte, showing a group of small irregular cells. See text. All drawings made from soaked material mounted in dilute glycerol.

veloped and indurated. The contents of one capsule were examined. The elaters proved exceedingly variable (fig. 9), just as is the case in the genera with elaters without spiral thickening (Proskauer 1951). Many are unicellular, most were composed of two, and a few of three cells. The pale yellow

spores vary from 50–72 μ (mostly ca. 57 μ) in maximum diameter. They bear a marking of fine dots on all faces, and on the outer face (fig. 13) in addition some large knobs. The latter appear to be blisters in the outer spore coat. No subdivision could be seen within the spores, which are probably unicellular.

The organism is the most luxuriant member of the Anthocerotales which has come to my notice. Stephani (1916) placed it in the genus *Megaceros*. This broaches the difficult problem of the definition of the genera *Megaceros* and *Dendroceros*. Further study may substantiate my impression that *Dendroceros*, as now defined, is composed of two parts, only one of which is closely related to *Megaceros*. The development of a unistratose wing is commonly found in species of *Megaceros*, at least under certain growth conditions. In such instances, according to current definitions, the only difference between the genera appears to be that *Megaceros* has broadly radiating thalli, and *Dendroceros* strap-shaped ones.[3] On this basis I tentatively place the present plant in the latter genus, as *Dendroceros endiviaefolius* (Mont.) comb. nov. The plant may be related to, or even identical with, *Anthoceros giganteus* Lehmann et Lindenberg (1832; syn. *Megaceros giganteus* [Lehm. et Lindenb.] Stephani, 1916) from New Zealand, a plant which with the other members of the Anthocerotales described by these authors needs re-examination.[4] Schiffner (1889) already remarked on the similarity of these two species. Gottsche et al. (1844–47) noted that *A. giganteus* is like *Dendroceros crispus*, only three times as large. It seems odd that they did not place it in *Dendroceros*. Goebel (1906) supplied a brief description and he also remarked that it belonged to *Dendroceros*. It is here placed in that genus, as *Dendroceros giganteus* (Lehm. et Lindenb.) comb. nov. It should, however, be noted that Montagne saw part of the type of *D. giganteus* in connection with his earlier study of *Anthoceros fuciformis* (1843).

7. **Anthoceros cichoraceus** Mont. = ***Dendroceros cichoraceus*** (Mont.) Steph. The plant was described by Montagne in 1845a. Later he placed it in *Dendroceros* (1850, p. 322), although the combination *D. cichoraceus* must technically be credited to Stephani (1909). The type material was collected by Gay on mossy *ground* somewhere in southern Chile. Two packets exist in the Montagne herbarium, apparently containing the same material. One bears the additional inscription "Gottsche n. 403"; it is apparently a duplicate containing a few small pieces. The other packet is here considered as

[3] The chloroplasts do not provide a constant generic character; I have collected a *Megaceros* in the upper Amazonas area which has in each cell a single chloroplast with 'pyrenoid'. Similarly, unicellular spores have been reported for several reputed species of *Dendroceros*.

[4] The description supplied by Khanna (1944) does not appear to belong to this species.

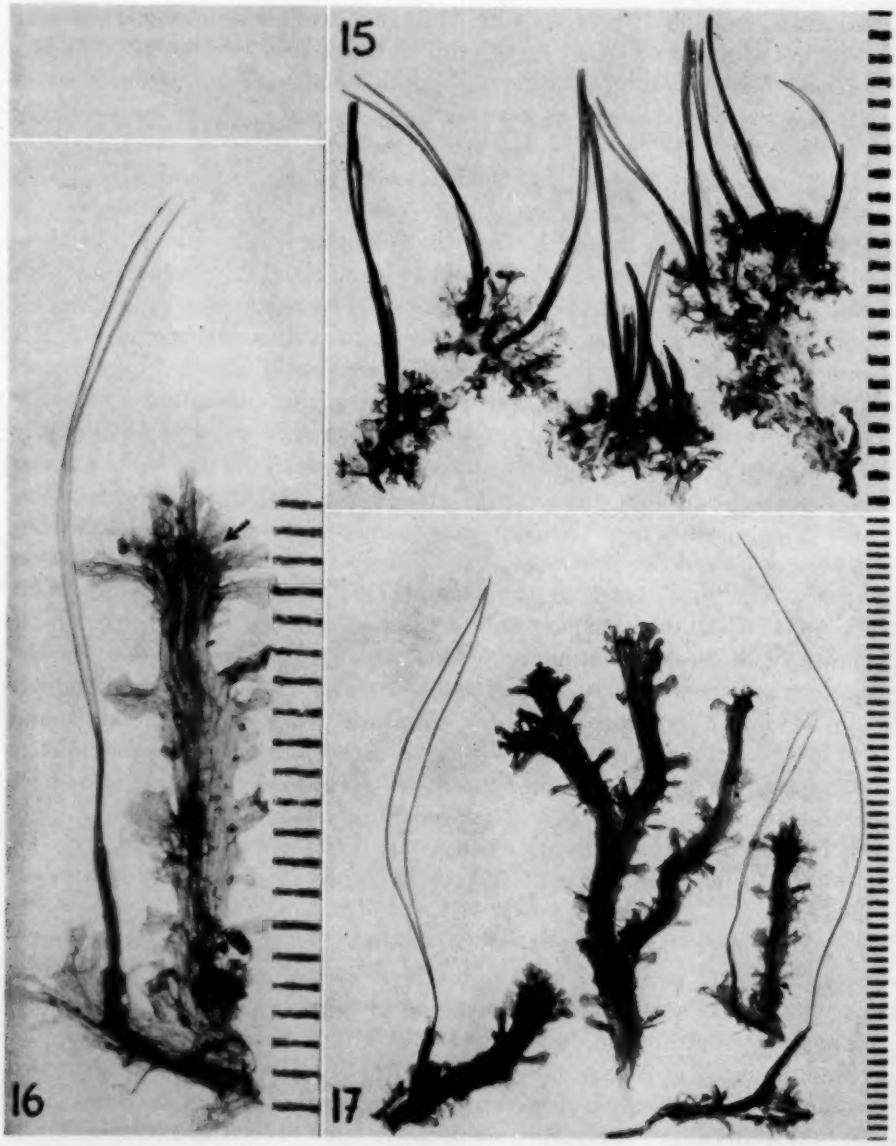


FIG. 15. *Dendroceros cichoraceus*. Bulk of type material. FIGS. 16. & 17. *Anthoceros fuciformis*. FIG. 16. Type material specimen with both sporophyte and male organs. The antheridial cavities, behind the growing point marked by arrow, are still closed. FIG. 17. Part of type material. All scale intervals 1 mm. All photographs in this paper by Mr. Victor Duran. The plants in the photographs are shown in the soaked condition and illuminated mainly by transmitted light.

containing the type material proper, and the following information was obtained from it. The bulk of the lectotype material is shown in figure 15, which may serve to illustrate the gross morphology, and supply the dimensions of the thalli. The plants are monoecious; antheridial cavities were seen in the two plants on the right side of the photograph. Intact antheridia were not found. The unistratose wings of the thallus show only a few perforations, most of these probably were formed by a breakdown of single cells, rather than the formation of an intercellular space through rounding off of cells. The sheaths (involucres) vary from about 3.5 to 7 mm in length. They bear a few small scattered surface outgrowths; without a search for these outgrowths one might easily describe the sheaths as smooth. The longest sporophyte (plant on left side of the photograph) measures 14 mm in height, but it is broken off and must have been longer. In the moist condition, as shown in the figure, the valves are straight; when dry they show some twisting. Certain of the capsules had dehisced by two splits, the valves adhering apically (Proskauer 1948b), a point which Montagne (1850) stressed; it is not clear if this always is the case in this species, bearing in mind the heavy induration of the epidermal cells (fig. 6). This induration, also seen to the same extent in species of *Notothylas*, gives much greater rigidity to the valves here, compared with those of species of *Anthoceros* and *Phaeoceros*. The epidermis is light brown in colour, and lacks stomata. It is difficult to be certain of the exact details of the structure of the elaters. It appears that they are multicellular, the components having single spiral thickening bands. Figure 11 shows one 'typical' unbranched elater, and one that is branched. Their size is, of course, quite variable (Proskauer 1951); the unbranched elater in the figure represents about the norm. At the level of dehiscence the spores vary in size from about 52 to 72 μ (mostly ca. 65 μ) in maximum diameter.[5] Their shape is somewhat irregular. The spore coat is transparent and bears a covering of papillae (fig. 14). The contents are multicellular, but the limits of the cells are now hard to discern clearly. In one instance at least twelve cells were counted on one face of a spore. At the time of collection the spores appear to have been in active growth, they still contain much chlorophyll.

As Leitgeb and Waldner (1879, p. 38) pointed out, aborted spores are frequently found lying among the normal ones in this species. They held that in all instances two spores of a tetrad develop at the expense of the other pair, which aborts. These authors also mentioned the presence of unicellular spores among the multicellular ones. I have not been able to convince myself if these observations are correct or not, only a study of living or well preserved material can provide a satisfactory answer. Leitgeb and

[5] In how far measurements on the spores, germinating already in the capsules in this and related species, have a meaning must be determined by work on living material.

Waldner did not state the origin of their material, I presume it was part of the original collection.

I failed to find this plant in Chile, although I searched for it. It clearly is closely related to, or identical with, *Dendroceros crispus* (Sw.) Nees. Montagne (1845a) stated that he had a part of the type of *D. crispus,* and that it differed in several characters from his species. However, in the revised and detailed description of 1850 he pointed out that the two are very similar. Of the differences listed the only one to be considered seriously is that the thallus of *D. crispus* is supposed to be larger. It would be difficult to distinguish the two species according to the diagnoses in Stephani (1909, probably still a fairly reliable work; the diagnoses differ from those given in 1916), especially if one takes the limits of significance of the spore measurements into account. Howe (1934) described a specimen from the Galápagos Islands, and placed it in *D. crispus.* I have seen this material (Howell No. 255, Herb. Calif. Acad. Sc.). It appears indistinguishable from Montagne's plant, to which Howe oddly made no reference, except that the spores in the Galápagos material are somewhat smaller (Howe: 40–50 μ max. diam.; 52 μ in the only one I saw), but this is probably due to the relative immaturity of the sporophytes. Howe discussed the matter of the unreliability of the diagnoses and the lack of good differential characters for many of the species of this genus. There is a very strong presumption that *D. cichoraceus* is a synonym of *D. crispus,* and the same can be said of several other American species. My reasons for not placing the present species in *D. crispus* are that I have not seen the type material of the latter, and that so far no variability studies have been made on living plants in this genus.

8. **Anthoceros fuciformis** Mont. *Anthoceros fuciformis* (not to be mistaken for *A. fusiformis* Austin) was described by Montagne in 1843 from material collected by Gaudichaud in Réunion ('Bonite' Expedition). Representative plants from the type material are shown in figure 17. The strap-shaped habit of the plant, with the pinnately arranged lateral lobes, appears very striking. It may, however, not be the only, nor even the 'typical', phenotype of the species. Previously it has been shown that precisely this appearance is assumed by the 'moist etiolate' growth form of *A. husnoti* (Proskauer 1948a, p. 261), a plant which normally has shorter and broader segments. The present material lacks surface outgrowths, which may also be due to growth conditions. Large schizogenous cavities are present in the thallus, as is diagnostic for the genus (Proskauer 1951). The plants are monoecious; on one of the specimens both a sporophyte and immature antheridial cavities are present. They are found on different lobes (fig. 16), and the antheridia represent the next sexual phase following that which gave rise to the sporophyte. Certain other pieces bear numerous mature antheridial cavities, containing many antheridia; in one cavity at least 21

antheridia were counted. The antheridia have the 4-tiered bodies typical of the genus (Proskauer 1951). In the mature antheridia the bodies were rather uniformly 180 μ long.

The longest sporophyte measures 5 cm in length. It is probable that the valves are normally connected at their tips; they show marked spiral twisting when dry (Proskauer 1948b). The epidermis bears stomata (fig. 7), although not abundantly. The ordinary epidermal cells are rather long (some may even exceed 250 μ). The elaters are thick-walled and show the usual variable appearance, most are unbranched (fig. 10). The light-brown spores vary from 32 to 40 μ (usually 34 μ) in maximum diameter. They are ornamented with irregular knobbly outgrowths. The density and size of these knobs are variable, they are always smaller on the inner face of the spore than on the outer one. No clear triradiate ridges are apparent. The outer face of a 'typical' spore is shown in figure 12.

The type of *A. fuciformis* is very reminiscent of the remarkable material of a species of *Anthoceros* from the Cameroons, which has previously been mentioned (Proskauer 1951, p. 341 and fig. 33).[6] The gametophytes are similar. A fragment of the Camerunian plant showed that it too is monoecious; the antheridial bodies measure 170–180 μ in length. The sporophyte shows some points of difference. The spores have the same ornamentation, but appear paler and are consistently smaller (27–34 μ, mostly 30 μ). The main difference lies in the epidermis. The ordinary epidermal cells are shorter, and stomata are completely absent, although they show their usual development in the gametophyte. In one sporophyte, but not in others, a number of odd irregular clusters of small cells were present (fig. 8). A purely speculative explanation of the material is that it may have acquired, by hybridization or mutation, a gene causing suppression of stomatal development in the sporophyte. Such a gene is presumably present in *Megaceros, Dendroceros,* and *Notothylas.* The sporophyte with the odd clusters of epidermal calls might conceivably have been heterozygous for this gene. In practice such matters can only be decided if living material should become available. It is clear, however, that the material is very close to *A. fuciformis.* whatever its exact nature may be. Less is known about the species of Anthocerotales from tropical Africa than about those from any other part of the world. It may well be that *A. fuciformis* has a continuous distribution right across that continent, or that this is at least true of an '*Anthoceros fuciformis* complex', comparable to the numerous other species complexes known in the group, such as the *A. venosus-ravenelii* complex of eastern North America, and the *A. fusiformis* complex of the American west coasts.

[6] "Prope Jonje pagum . . . , Camerunia" 22. XI. 1891, leg. Dusén. (Herb. Univ. Wisconsin.) Identified as *A. pinnatus* Steph. (1887, type from St. Thomé) and accepted as such by Stephani. The plant does not agree too well with that author's own diagram of the St. Thomé plant (copy in Herb. Univ. Calif.).

SUMMARY

1. The type specimens of the species of *Anthoceros* created by J. F. C. Montagne are described.

2. The following additional observations were made: *Anthoceros dissectus* Mont. belongs to the Podostemaceae. *Dendroceros endiviaefolius* (Mont.) comb. nov. raises the problem of the generic differences beween *Megaceros* Campb. and *Dendroceros* Nees, a matter which is briefly discussed. The description of the sterile type material is augmented by an examination of fertile material collected by Dusén. The species is probably closely related to *Dendroceros giganteus* (Lehmann et Lindenberg) comb. nov. *Dendroceros cichoraceus* (Mont.) Steph. is very closely related to *D. crispus* (Sw.) Nees, and may even be a synonym of that species. *Anthoceros fuciformis* Mont. from Réunion appears closely related to certain anomalous material from West Africa, which lacks stomata in the sporophyte.

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AN ASPLENIUM PROTOTYPE OF THE GENUS DIELLIA

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The fern family Aspleniaceae is represented in the Hawaiian Islands by two genera: *Asplenium*, the cosmopolitan and very large assemblage of leptosporangiate ferns known as "spleenworts"; and *Diellia*, a unique and formerly controversial local genus. At present about 16 species of *Asplenium* are recognized in Hawaii, and 5 of *Diellia*, although in 1888 Hillebrand recognized many more (30 species of *Asplenium* and 8 of *Diellia*). Modern reduction in number of accredited species is not mere taxonomic "lumping"; it results from increased recognition of the variability of certain gross characters. Leaf cutting in such Hawaiian Aspleniaceae as *Asplenium Macraei*[1] *A. lobulatum*, *A. falcatum*, and *Diellia erecta*, may vary from once to thrice pinnate. (The latter phenomenon is by no means confined to Hawaiian Aspleniaceae, however, as can be shown in such New World species as *Asplenium radicans* and *A. auritum*.) Certain Aspleniaceae also have the capacity of becoming markedly dwarfed under rigorous environmental factors, or of becoming fully soriferous in the juvenile condition. In view of variability in these and other respects within species, due caution must be exercised in evaluating criteria used in dealing with the taxonomy of these ferns. It seems now that only detailed comparison of such "difficult" minute features as spores, scales, hairs, stipe structure, venation, and indusia, can provide really reliable guides to species delimitation and species relationships. Skottsberg (1942) used such characters as scales and spores, and thereby made considerable progress toward the understanding of Hawaiian species of *Asplenium*.

This paper deals with the Hawaiian members of the group of simply pinnate spleenworts of relatively small or moderate stature with dark-maroon to black, lustrous petioles—the so-called "black-stemmed" rock spleenworts. It deals particularly with the status of an unusual and misinterpreted member of this group, a species which appears to have special phylogenetic significance in connection with the origin of the genus *Diellia*. This species, *Asplenium leucostegioides*, is known at present from only three herbarium sheets, and it was reduced to synonymy by Christensen (1934) under another Hawaiian species. In view of the remarks of the foregoing paragraph it might seem unwarranted now to "resurrect" this species. But careful examination of such minute characters as those listed shows that upholding *A. leucostegioides* as distinct is amply justified.

[1] References for taxonomic names used in this paper are in Christensen, C., Index Filicum 1906, and Copeland, E. B., Genera Filicum 1947.

This fern was noticed while I was making identifications of Hawaiian specimens of *Asplenium;* a single collection (fig. 2) was observed in the series of *A. normale.* Subsequent assistance by Miss Marie C. Neal of the Bishop Museum enabled me to find an additional collection, this one identified as "*A. monanthes?*" Neither of these collections has heretofore been identified with *A. leucostegioides,* which was based upon a single collection of a juvenile plant quite different in aspect (Baker 1891). Through the kindness of the authorities of Kew, the type specimen was sent to me for comparison with the other collections. The handwritten annotation on the type is as follows: "Asplenium normale Don *pavonicum Brack. terminal bud with young leaves. Det. Carl Christensen 1930." The specimen, however, definitely does not represent a terminal bud; although an old leaf-axis attached at a peculiar angle to the stem gives the appearance of a persistent mother rachis as is so commonly seen in vegetatively produced individuals of *A. normale.* Examination of this leaf-axis reveals that the orientation of the original pinnae was away from, rather than toward, the rhizome of the plant. As will be discussed below, the young leaves possess very much larger pinnae than those in comparable plants of *A. normale.*

Asplenium leucostegioides, as this species will be redefined below on the basis of the three known collections, has not been rediscovered in the Hawaiian Islands for over seventy years. What the precise habitat of this fern was can only be conjectured. F. L. Clarke, who was the collector of two of the three specimens, was living at the time they were found at Makawao, east Maui (letters to Asa Gray, preserved in the Gray Herbarium). In the same year, 1879, D. D. Baldwin took the third specimen. Both Clarke and Baldwin collected ferns in that area, and although some of their specimens came from the vicinity of Olinda, a few miles away and higher up on the northeast slope (about 3500 ft.) of Haleakala Crater, the altitude of 2500 feet given on Baldwin's collection suggests that the species may have been found closer to Makawao. Resembling as it does such local species as *A. normale,* as well as species of *Diellia, A. leucostegioides* might easily be overlooked, and this may account for the fact that only three collections have been made. However, for future field efforts to study this rare plant, the Makawao area of east Maui should be thoroughly examined.

In making this study I am indebted for help in various ways to Dr. Reed C. Rollins and the staff of the Gray Herbarium. I also wish to acknowledge the kindness of Miss Charlotte Pratt in preparing the photograph. The investigation was made while I was a Research Fellow at the Gray Herbarium.

The following description of *Asplenium leucostegioides* redefines the concept of this species, stressing the mature form of the plant, which has heretofore been undescribed.

Description. A fern of small to moderate size; the fronds tufted (fig. 1), five or more photosynthetic ones per rhizome and at least one remain-

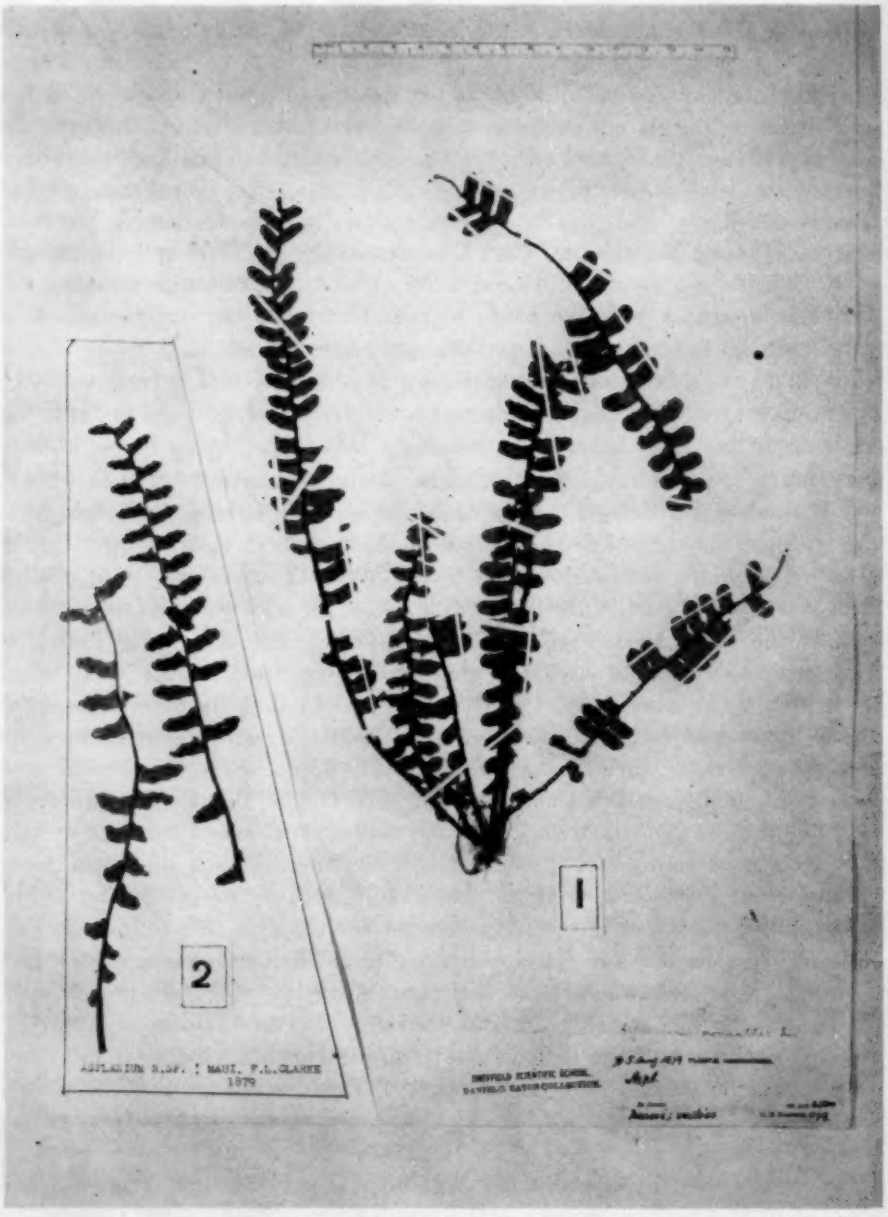


FIG. 1. *Asplenium leucostegioides*, Maui, *D. D. Baldwin.* FIG. 2. *A. leucostegioides*, Maui, *F. L. Clarke.*

ing dead rachis; rhizome erect, short, up to 0.8 cm. tall, and 0.6 cm. in diameter including the surrounding dead stipe bases; rhizome scales large, conspicuous, long-deltoid in outline and attached along a basal line, 2.0–3.5 mm. long, 0.5–1.2 mm. broad near base, brownish, clathrate, the scale margins with some sharp lateral emergences comprising several cells, the central cells with somewhat thicker and darker lateral walls (figs. 3 and 4); fronds once pinnate, with very short but robust stipes, the frond outline linear-lanceolate, 20–30 cm. tall (estimated), 2–3 cm. broad in the middle; stipes and rachises slightly ridged above, purple-maroon, shiny, sclerified at the surface, and invested with more or less numerous fibrils (of the form shown in figs. 5 and 7) and some glandular hairs, the latter trichomes tending to fall at maturity but remaining at the pinna bases beneath; stipes 0.9–1.2 mm. in diameter, 1.5–3.0 cm. long, a cross section of the stipe base showing a single bundle with two oblique xylem strands fused into the outline roughly of a wide-based Y; pinnae 25–35 pairs (est.), sessile, attached at a point or adnate for 1–2 mm.; lower pinnae conspicuously but gradually reduced in length, down to one-fourth as long as the middle pinnae, and ear-like (figs. 27 and 28); middle pinnae (figs. 18–20, 23–25) subremote to approximate, simple, 0.9–1.8 cm. long, 0.5–0.7 cm. broad, truncate at base, with pointed anterior auricles which tend to overlap the rachis near the point of pinna attachment; pinna tips rounded or rounded-truncate; pinna margins perfectly entire or shallowly and smoothly undulate, or rather deeply and coarsely undulate (fig. 19); frond tip apparently gradually and symmetrically reduced; blades stiff, subcoriaceous, dull grey-green; mesophyll of spongy parenchyma (no palisade parenchyma present); epidermal cells large (in relation to those of nearly related species), oblong or squarish with undulate lateral walls; epidermis provided with more or less persistent but scattered glandular hairs of 3 cells (rarely more), the upper two of the cells globular, and the basal cell urn-shaped, the glands adpressed against the laminar surface (figs. 54–57); venation entirely free, with 3 or 4 lateral vein pairs arising remotely on each side of the costa, those in the lower half of the pinna forked once (at the pinna base occasionally more), those of the distal part of the pinna simple; sori asplenioid, 2–4 pairs per pinna, medial to supramedial, short, the indusia 0.9–2.0 mm. long, 0.5–0.7 mm. broad, oblong to oblong-lunate, entire to slightly jagged-margined (fig. 58); the indusial cells in one layer except at the attachment, the cell walls undulate; individual indusial cells relatively large and squarish or oblong in over-all outline, 80–90 μ in average greatest dimension; sporangia large (figs. 59 and 60), the capsules between 300 and 350 μ long before dehiscence; sporangial stalks of one cell row; annulus with 19 (15–22) thickened cells, and directly connected to the stomium which comprises 3 or 4 somewhat thickened cells; spores large, dark-brown, translucent to opaque, elliptic, the exospore 38–45 μ long, 25–35 μ across, and covered with a thick and jagged perispore layer; young sporophyte with relatively large-bladed cuneate to oval simple fronds; early pinnate fronds with large, broad terminal "pinna"; gametophyte with 2-celled adpressed marginal glandular hairs.

Material examined. TYPE; "Asplenium 42 + n. sp., E. Maui, Haihei [the spelling of this word is undetermined, and may be differently interpreted], Sandwich Islands, G. E. Davenport, 7/82, No. 1" (according to

Baker, 1891, this was collected by F. L. Clarke in 1879); "Vallies, alt. ped. 2500, Aug. 1879, D. D. Baldwin no. 5" (Scheffield Scientific School, D. C. Eaton Collection, Yale—at present on loan to Bishop Museum); "Maui, F. L. Clarke, 1879" (Gray Herbarium).

Relationships. Attention must be called to this hitherto taxonomically confused and overlooked Hawaiian fern because of its surprisingly numerous close resemblances to the genus *Diellia*. The latter genus has been the focus of considerable taxonomic and morphological attention since its original description by Brackenridge in 1854. In its varied subsequent career in taxonomic interpretation *Diellia* was associated by authors most notably with three different groups of ferns, two of these, the lindsaeoid and davallioid ferns, because of its sori which are similarly marginal in position (figs. 29–34). Copeland (1929) first gave evidence that *Diellia* is related actually to the Aspleniaceae, ferns with usually superficial sori, and recent studies by the writer yielded considerable additional data (1952) which support his conclusion. Christ in his book "Die Geographie der Farne" (1910) had called *Diellia* the most striking genus of ferns in the Hawaiian flora, but he emphasized at the same time the confusion which surrounded its interpretation. The recent studies of the morphology, anatomy, and cytology of *Diellia*, however, have supported not only Copeland's conclusion regarding the asplenioid affinities of the genus, but have also indicated ancestry specifically among the familiar "black-stemmed" spleenworts. This group of spleenworts, the species of which occur primarily in rocky and somewhat arid habitats, may be exemplified by the familiar "maidenhair spleenwort," *A. Trichomanes*. Besides the latter, the Hawaiian flora includes *A. monanthes* and *A. normale* (the two species to which the three known collections of *A. leucostegioides* have been referred).

Although apparently derived from this group of *Asplenium*, the genus *Diellia* is a highly diversified genus within itself, which shows evidence of strong evolutionary trends in several directions. But it is now believed that the polymorphic and most wide-ranging of its species, *D. erecta*, most nearly resembles the presumed ancestral type within the Aspleniaceae and it seems also to be the most generalized morphologically of the known forms of *Diellia*. In the discussion of the morphology which is to follow it is therefore primarily *D. erecta* in the broad sense which is to be kept in mind for comparative purposes, rather than such specialized types as the finely divided-leaved *D. Mannii* of Kauai, or the much-reduced *D. unisora*, or the very scaly, reticulate-veined (fig. 33) and pale-stiped *D. falcata*, both of Oahu.

The first of the characters to be compared among these ferns is the general habit of the plant. Most of the populations of *Diellia* (cf. Smith, 1934, pl. 1–7) comprise plants of considerably larger size than is charac-

teristic of typical rock spleenworts, but those plants of *Diellia* which have smaller stature, more comparable to typical specimens of *A. Trichomanes, A. monanthes, A. normale, A. resiliens,* and *A. platyneuron,* usually display more robust stipes than these. *A. leucostegioides* resembles *Diellia* closely in this respect. The typical rock spleenworts tend to retain numerous stiff dead rachises in a persistent tuft about the rhizome; but such a condition apparently does not occur in most of the populations of *Diellia,* nor is it found in *Asplenium leucostegioides.* Of species of *Diellia,* only the peculiar *D. unisora* (Smith, op. cit., pl. 6, fig. B; Wagner, 1951, pl. 2) has the narrow, wiry type of leaf axes and retention of numerous dead rachises that are found among the typical rock spleenworts. The latter conditions may be due merely to the relatively greater amount of mechanical sclerenchyma in mature stipes of smaller diameters. The large leaf-axis diameter of *Asplenium leucostegioides* in combination with the lack of wiriness and retention of old axes makes an ensemble of peculiarities of the plant habit which immediately remind one of *Diellia,* rather than typical rock spleenworts.

The internal anatomy of the leaf axis of *Asplenium leucostegioides* shows a single Y-shaped strand in cross section, as is characteristic of all species of *Diellia* and of those of *Asplenium* which belong to the rock spleenwort group. (The remaining species of *Asplenium* show two vascular strands clearly separated from each other.) The internal anatomy of the leaf axis of *A. leucostegioides* thus confirms its relationships as being in the *Diellia-Asplenium Trichomanes* complex.

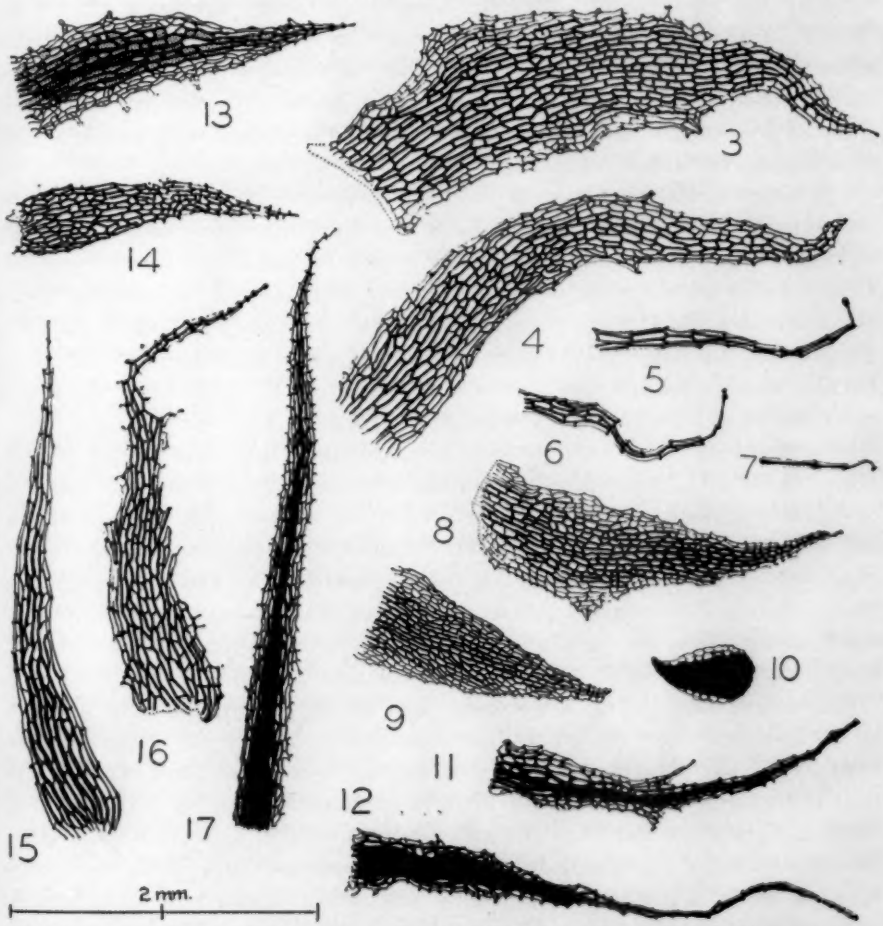
The presence and nature of the trichomes borne on the stipe and rachis of *A. leucostegioides* provide a suggestive point of comparison. In this species there are clathrate fibrils (figs. 5–7) and glandular hairs (in form like those of the lamina shown in figures 54–57) which persist along the length of the leaf axis, and at the lower part of the stipe there are conspicuous, broad, clathrate scales as well. In one of the collections of mature specimens (Clarke, Gray Herbarium) these trichomes are rather sparse along the rachis, appearing mainly as narrow scales or fibrils, and persisting most abundantly around the pinna bases on the abaxial side; in the other (Baldwin, Bishop Museum), however, the leaf axes are densely invested by these structures. Both of these conditions are commonly seen among the species and forms of *Diellia,* especially in the populations of *D. erecta* near Makawao, Maui, *D. erecta f. Alexandri* at Olinda, Maui, and Kokee, Kauai, and practically all the populations of *D. falcata.* Either condition, however, is completely unknown to me in matured fronds of the typical rock spleenworts. Only the genus *Ceterach* (which also seems to be of this general circle of affinity) has species with such scaly rachises, although its scales are of a different type.

The ridges on the adaxial side of the rachis in *Asplenium leucostegioides*

are indistinct because of the tendency of the leaf axis to be somewhat twisted. In *Diellia* the ridges seem to be more distinct—one running up each side of the top of the rachis—because the rachis is straighter. In the more typical rock spleenworts this feature tends to be characteristic of species. In *Asplenium Trichomanes, A. heterochroum,* and *A. resiliens,* for example, the ridges are formed of non-pigmented cells, so that there appear to be green or white "flanges." The laterally compressed rachis of *A. normale,* on the other hand, possesses a black-ridged adaxial surface, the ridges being separated by an unusually deep groove. Comparison of the nature of the ridges is at present inconclusive regarding *A. leucostegioides* and may be further clarified if more and better materials of this species are obtained in the future. The color of the surface layers of the leaf axis in *A. leucostegioides*—a deep maroon-black—can be matched equally in *Diellia* (for example, *D. erecta, D. erecta* f. *Alexandri,* and *D. Mannii*) and in *Asplenium* (*A. Trichomanes* and *A. monanthes*).

In regard to the rhizome scales of *A. leucostegioides* it is necessary to consider the size of the plants being compared. It has been found in a study of *Diellia* (Wagner 1952) that small plants, whether juvenile or dwarfed, have smaller scales than do the mature or full-sized plants of a given population, although this does not apply to glandular hairs. In *Diellia* the rhizome scales are remarkably diverse for such a closely knit genus; the two most specialized scale types in this genus are those of *D. falcata* which are "plate-like" and very large (up to 7.0×2.7 mm.) and dark-maroon, and those of *D. unisora* which are extremely small (averaging 0.8×0.4 mm.), very thick, and entirely jet-black except for a narrow pale margin (fig. 10). However, the scales of the remaining three species of *Diellia*—*D. erecta, D. Mannii,* and *D. laciniata*—are rather uniform, being clathrate (the lateral cell walls heavily thickened by secondary depositions but the facial walls hyaline and transparent), and having a broad cell plate (figs. 8 and 9). The central cells of these scales, which seem to be the more primitive type, generally have thicker secondary wall layers than do the lateral cells. The scales of *Diellia* in general tend to be curled rather conspicuously inwardly toward the axis. In comparison with typical "black-stemmed" rock spleenworts, scale size in *Diellia* is larger. The average of largest scale sizes measured in *Diellia* species is 4.0×1.4 mm., but the average plant size is larger in *Diellia* than in *Asplenium leucostegioides.* In the latter species the largest scales (figs. 3 and 4) are 3.5×1.2 mm. which compares closely with plants of equal size (fig. 8) of *Diellia,* and is larger than in populations of small or dwarfed plants (fig. 9). The structure of the scales in *Asplenium leucostegioides* is like that in Diellia, and there is also a strong tendency for the scales to curve adaxially, so strong in fact that it is difficult to flatten them in mounted slide preparations.

Such robust scales as those described above do not occur in typical rock spleenworts: the scales of *A. Trichomanes* (fig. 17), *A. heterochroum*, and *A. resiliens* (figs. 11 and 12), for instance, are very narrow, even though



FIGS. 3–7. *Asplenium leucostegioides, Baldwin.* FIGS. 3 and 4. Rhizome scales. FIGS. 5–7. Rachis fibrils. FIG. 8. *Diellia erecta,* Puu Kolekole, Molokai, *Wagner 5810.* FIG. 9. *"D. pumila,"* Lahainaluna, Maui, *Wagner 5416.* FIG. 10. *D. unisora,* Pohakea Pass, Oahu, *Wagner 5800.* FIGS. 11 & 12. *Asplenium resiliens,* Coahuila, Mexico, *Johnston & Muller 613.* FIG. 13. *A. normale,* Kauai, *Wagner 3220.* FIG. 14. *Camptosorus rhizophyllus,* West Virginia, *Gilbert 272.* FIG. 15. *Asplenium Pringlei,* Mexico, *Pringle 1837.* FIG. 16. *A. platyneuron,* New York, *Carey s.n.* FIG. 17. *A. Trichomanes,* Maui *Wagner 5221.*

large plants of *A. Trichomanes* may have scales which reach 5 mm. in length. The scales of the even larger plants of *A. monanthes* may become still longer, up to 6 × 1 mm., but the same proportion of length to breadth of

the scale remains approximately as before. Scales of *A. normale,* on the other hand, tend to be relatively broader, but they are considerably smaller (fig. 13) in over-all dimensions than those of *A. leucostegioides* in plants of comparable size, being only 2.5×0.7 mm. (cf. also Skottsberg 1938, figs. 33–35). A plant of *A. platyneuron* one and one-half times the size of the known material of *A. leucostegioides* will have scales (fig. 16) which are considerably smaller. Thus the scales of *A. leucostegioides* are most like those of *Diellia,* and this fact, combined with the conspicuous development of scales on the stipes, enhances the close resemblance.

In comparisons of pinnae of the fern under discussion with those of its presumed relatives it is the ensemble of pinna-characters which is most significant, as will become evident. The pinnae of *Asplenium leucostegioides* (figs. 18–28) show the following distinctive combination of features: they are sessile to adnate, with truncate bases that tend to overlap the rachis; the margins are smooth, and straight or undulate; the veins are free and unusually widely spaced; and the basal pinnae (figs. 27 and 28) of the blade are reduced so that they appear as short, rounded auricles.

The bases of the pinnae provide a special point of similarity to *Diellia* (figs. 29–34). In that genus the pinnae are similarly attached at a point, or adnate up to 1 or more millimeters on the rachis[2]. The outline of the pinna base in *Diellia* is usually truncate, and is often more or less convex near the sides of the attachment, so that a laminar flap overlies the midrib along an axial distance of 1–3 mm., exactly as in *Asplenium leucostegioides.* Such pinna bases are rare among other rock spleenworts, however. In *A. resiliens, A. monanthes* (figs. 38–40), *A. normale* (figs. 35–37), *A. formosum* (figs. 46 and 47), and *A. harpeodes* (figs. 50–53), the pinnae are long- or short-stalked, and the outline at the pinna-base is usually an obtuse or acute angle. In *A. Trichomanes* (fig. 41) and *A. heterochroum* (figs. 42–45), the pinnae, being oval-oblong, have a different outline than those just listed, but their bases run out from the attachment similarly at an angle; in these two species the pinna stalks remain after the pinnae have fallen, as decurrent pedestals along the rachis. Only the rather isolated *A. platyneuron* (figs. 48 and 49), a species with leaf dimorphism—the fertile fronds tall and erect, and the sterile short and spreading—has the truncate pinna bases of *A. leucostegioides,* but, as will be seen, it differs in a number of the other characteristics of its pinnae.

The smooth, straight or undulate pinna margins of *A. leucostegioides* are closely matched in the once-pinnate populations of *Diellia.* (The majority of forms in this genus have only this degree of cutting.) *D. falcata,* a species specialized also in other respects, tends to have low, rounded projections of the laminar margins at the positions of sori; and the rare, dis-

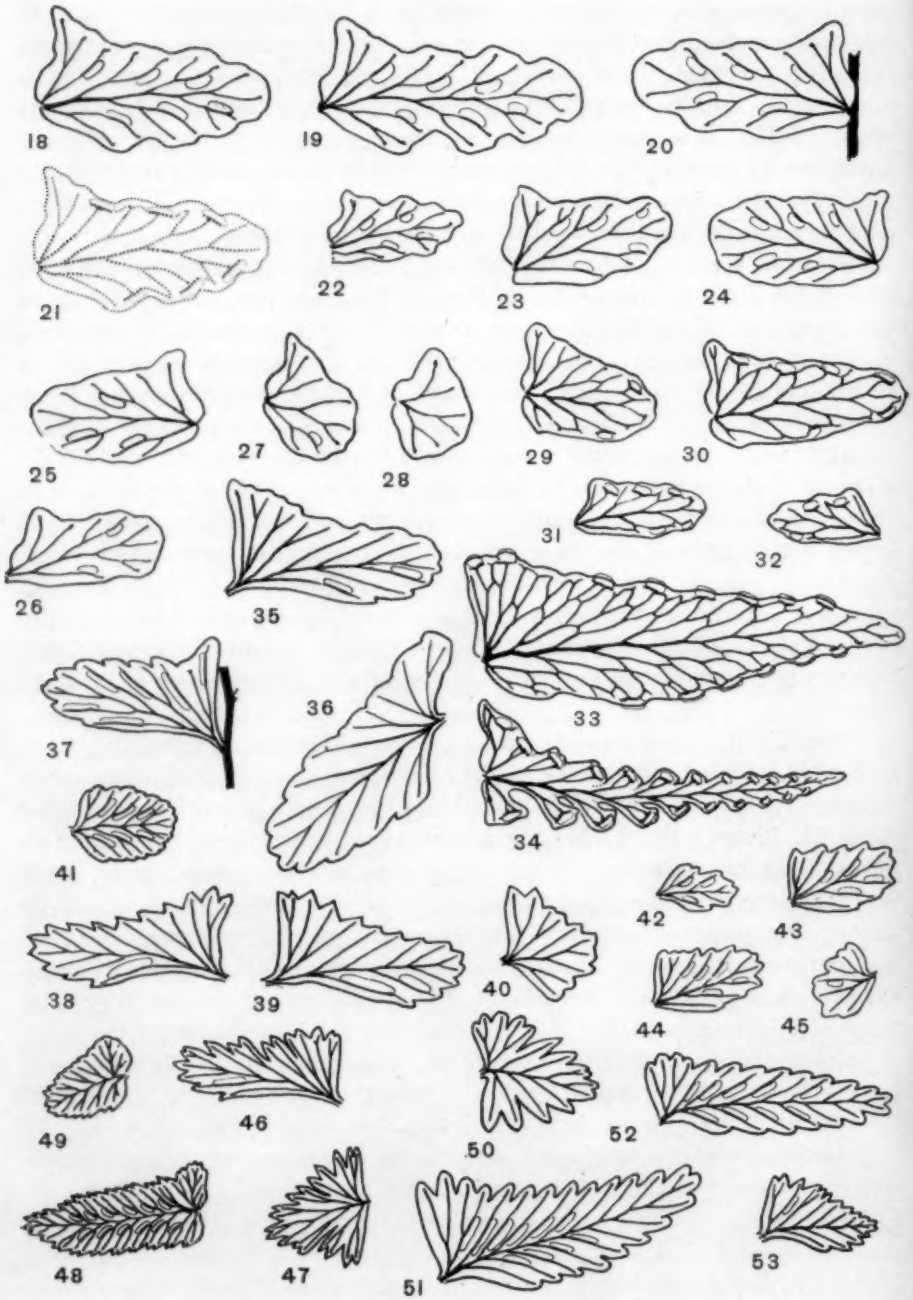
[2] This is a characteristic of *Diellia* which has not been previously emphasized.

sected species and varieties of *Diellia* such as *"D. Alexandri"* and *D. Mannii* have deep sinuses between the sori. The entire-margined *Asplenium leucostegioides* is different, however, in this respect from comparable plants of any of the "black-stemmed" spleenworts. ***A. resiliens*** and ***A. heterochroum*** (figs. 42–45) have more or less shallowly dentate pinna margins; *A. Trichomanes* has usually crenate margins (fig. 41); *A. platyneuron,* minutely but distinctly bidentate margins (figs. 48 and 49); and *A. monanthes* and *A. normale,* coarsely toothed margins (figs. 35–40).

Dwarfed basal pinnae, which become progressively reduced toward the base of the blade (pinnae reduced down to one-fourth or less as long as the middle pinnae of the blade), occur commonly in these ferns. The situation of auricle-like basal pinnae occurring in *A. leucostegioides* (fig. 28) is found alike in such species as *Asplenium resiliens, A. platyneuron,* and *A. formosum,* as well as in *Diellia Mannii, D. falcata,* and the majority of other populations of *Diellia.* Such a condition is not so marked, however, in *Asplenium Trichomanes* and *A. heterochroum* (fig. 45); in the latter two species the basal pinnae are generally not reduced more than one-third in length and they tend to be spaced very distantly from each other. In *A. monanthes* (fig. 40) the situation is rather like that in *A. Trichomanes,* but that of *A. normale* is distinctive. In *A. normale* the basal pinnae are usually not at all or very little reduced, but are strongly modified, being characteristically asymmetrical and usually pointed downward at a strong angle (fig. 36).

Individually these characteristics of the pinnae of *Asplenium leucostegioides* may be found in other ferns of assumed relationship but taken together the combination cannot be matched as a unit in any of the related Asplenia. Instead, the combined peculiarities of outline and attachment of pinna bases, the nature of the pinna margins, and the basal pinnae, mark this species as most closely resembling *Diellia.* The latter genus, minus its unique commissural secondary veinlets, would have a vein pattern much like the present species. In addition to these points of resemblances, the over-all pinna outlines in *Asplenium leucostegioides* and in various populations of *Diellia* are much alike; but they are unlike those in such species of spleenworts as *Asplenium normale, A. monanthes,* and *A. Trichomanes.*

The sorus of the present species is typical structurally of *Asplenium,* the receptacle being linear along one side of (usually) the anterior veinlet if the subtending vein is divided, and the indusium one cell layer in thickness. The outline of the fertile vein is shown by the black line in figure 58. The ontogenetic origin of this sorus is undoubtedly superficial as it is in *Asplenium,* and as was recently found to be the case in *Diellia* (Wagner 1952). The indusial cells have strongly undulate walls as in the other typical rock spleenworts and as in *Diellia,* but the dimensions of indusial cells in the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present species average larger (90 μ in the greatest dimension in the Clarke specimens, and 80 μ in the Baldwin plant) than those of any of the other plants studied (40–70 μ). For comparative purposes the length-breadth ratio of the sorus has been found to be significant in this case. The sorus of *Asplenium leucostegioides* is quickly distinguishable from sori of comparable plants of *A. Trichomanes* by its relative shortness. The indusium of adult fronds of *A. leucostegioides* is generally about three times as long as broad, although it varies from once to nearly four times. The juvenile but fertile plant of *A. leucostegioides* has extremely short sori. The indusia of the other Asplenia tend to be relatively narrower: in *A. Trichomanes*, for instance, it is usually 4–10 times longer than broad, and in *A. normale* and *A. monanthes*—ferns with larger sori—the relationship is approximately the same. The length-breadth ratio of the indusium in *A. leucostegioides* is like that in unfused sori of *Diellia*, where it is also usually 3: 1. The seemingly discrete and often much longer sori of "*D. Alexandri*" are actually "schizostegeoid" (Wagner 1949) in that they are interrupted marginal coenosori comparable to the remarkable sori of another Hawaiian endemic fern, *Pteris Lidgatei*. It is an odd fact which heightens the similarity of the present plant to *Diellia*, that, ***without change in sorus length***, re-orientation of the sorus of *Asplenium leucostegioides* into the marginal position nearly parallel with the pinna edge as in *Diellia* could take place in evolution as shown in the diagram in figure 21 (cf. fig. 18). Conversely, without its commissural veinlets and with its sori formed in the position of *Asplenium*, *Diellia* would be difficult to distinguish from *Asplenium leucostegioides*. In the common rock spleenworts (cf. *A. Trichomanes*, fig. 41; *A. normale*, fig. 37; and *A. monanthes*, fig. 39), however, soral length roughly equals or exceeds two of the intervals between vein termini. Therefore, re-orientation of sori of these species into the *Diellia* pattern would be impossible as the individual sori are relatively too long. The problem of soral length in assuming the origin of *Diellia* from rock spleenworts can now be resolved by observing the size relationships in *A.*

FIGS. 18–28. *Asplenium leucostegioides*, pinna structure. FIGS. 18–22. *Clarke* specimen (GRAY). FIG. 21. Pinna drawn diagrammatically with the sori re-oriented as in *Diellia*. FIG. 22. Upper pinna. FIGS. 23–28. *Baldwin* specimen. FIGS. 23–25. Middle pinna. FIG. 26. Upper pinna. FIGS. 27 and 28. Lower pinnae. FIGS. 29–32. *Diellia erecta*, Puu Kolekole, Molokai, *Wagner 5810*, pinnae from different positions from base to near apex of frond. FIG. 33. *D. falcata*, Kaawa Gulch, Oahu, *Wagner 5461*. FIG. 34. "*D. Alexandri*," Olinda, Maui, *Wagner & Bonsey 5320*. FIGS. 35 & 36. *A. normale*, Kauai, *Wagner 5462*. FIGS. 38–40. *A. monanthes*, South Africa. FIG. 40. Basal pinna. FIG. 41. *A. Trichomanes*, Virginia, *Wagner 2023*. FIGS. 42–45. *A. heterochroum*, Puerto Rico, *Wagner s.n.*, pinnae taken at different levels from near apex to the base of the blade. FIGS. 46 & 47. *A. formosum*, Guatemala, *Bartlett 12179*. FIG. 47. Basal pinna. FIGS. 48 & 49. *A. platyneuron*, New York, *Carey s.n.* FIG. 49. Basal pinna. FIGS. 50–53. *A. harpeodes*, Jamaica, *Maxon 10565*, pinnae taken at progressively higher levels from near the base of the blade toward the tip.

leucostegioides, where the sori are equal in length to *one of the intervals between veinlets.*

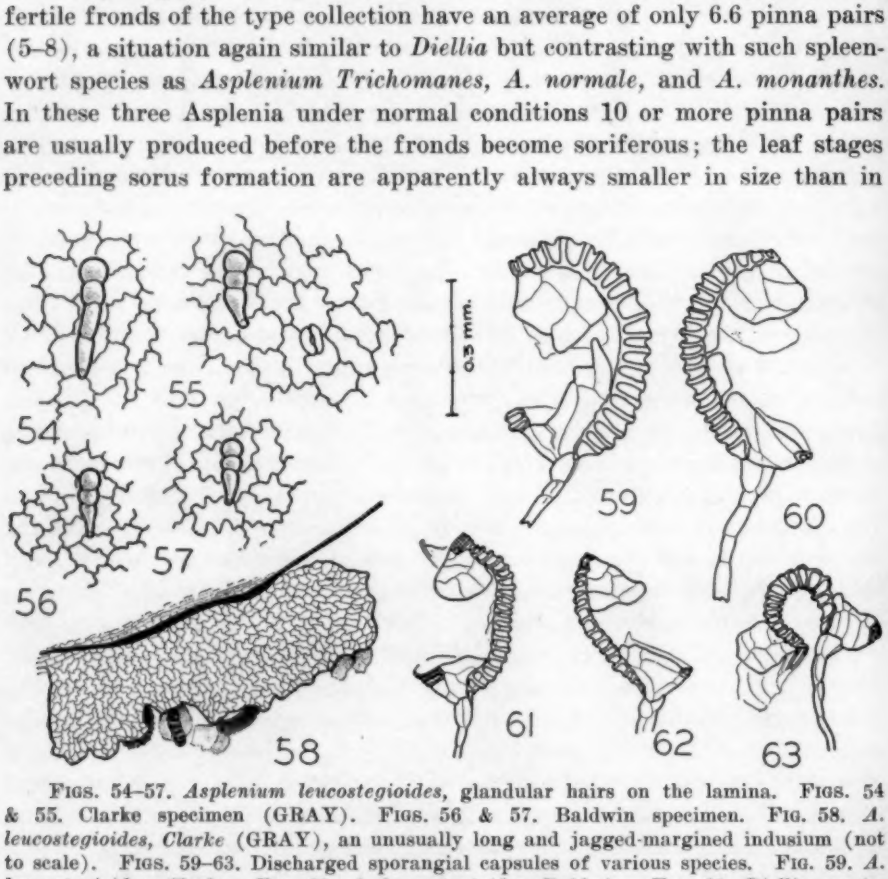
The spores of all the rock spleenworts as well as those of *Diellia* which I have examined tend to be rather small, 30–35 μ long, and 20–25 μ broad. The spores in the present species, however, are conspicuously larger, being over 40 μ long and 30 μ broad. The large size is probably correlated with large cells observed in the prothallia, indusia, sporangia, and elsewhere, and may possibly indicate polyploidy. The specific structure of the spores of *Asplenium leucostegioides* nevertheless is the familiar one among all the Diellias and rock spleenworts, although the very dark pigmentation is not common in such spleenworts as *A. monanthes, A. normale,* and *A. Trichomanes,* the spores of which are usually very pale-tan and nearly transparent. The species of *Diellia* and *Camptosorus* do, however, tend to have deeply pigmented spores.

Numerous but inconspicuous fragments of the gametophytes of a fern and a species of Jungermanniales were found on the rhizome of the type collection of *Asplenium leucostegioides.* The fern gametophytes represent, with considerable likelihood, the haploid generation of this species. The presence on the sides of the same rhizome of a young sporophyte, obviously of *A. leucostegioides,* not only tends to corroborate this view, but suggests also that the specimen must have been taken at a spot where this species of fern was reproducing abundantly (perhaps on a soil bank or on a boulder along the side of a gulch, as is commonly the case with the genus *Diellia,* illustrated by Wagner 1950). The fragments on the rhizome of the type specimen were first taken to be detached scales, but the cellular pattern and dark-green color betrayed their identity as gametophytes. Some of the pieces were carefully removed and placed in a weak solution of sodium hydroxide for 12 hours, a treatment which expanded the cells to their normal shape and size. On most of the fragments the margins showed no glandular hairs, or only the positions where these had been broken off. But fortunately three of the small fragments showed marginal hairs clearly, these embedded in a mucilaginous substance which was flattened against the prothallial margin. A large dark red-brown spot in each mucilaginous mass indicated the presence of a gland-cell, and further clearing in warm lactic acid and bleaching in sodium hypochlorite showed the hairs to comprise two cells, a cylindrical or slightly inflated-cylindrical stalk-cell, and a large, globular gland-cell. When the gametophytic fragments later were dehydrated in alcohol and mounted permanently in diaphane, they were compared with similarly prepared gametophytes of *Diellia falcata* and *D. erecta* (*"D. Alexandri"*) on cell size. The marginal cells were measured lengthwise along the margin and gave the following figures: *D. falcata,* 38 (30–50) μ; *"D. Alexandri",* 36 (20–50) μ; and *Asplenium leucostegioides,*

56 (40–65) μ. The same ratio of cell size between *Diellia* and this *Asplenium* held also for the glandular hairs. Although considerably larger, the hairs appear to match exactly those of *Diellia* in their structure. (Whether chloroplasts were originally present in the stalk-cell could not be determined.) The glandular hairs of *Diellia* had been heretofore considered somewhat unique in their structure among those known in fern gametophytes, but now the four conditions of trichome development in the Aspleniaceae for the haploid generation may be listed and exemplified as follows: gametophytes entirely glabrous (except for the rhizoids of the ventral surface)—*Asplenium Trichomanes, A. viride,* and *A. marinum;* gametophytes with "papillate" margins (i.e., with single-celled protuberances of characteristic shape)—*A. Adiantum-nigrum, A. Ruta-muraria, A. septentrionale, Camptosorus,* and *Phyllitis;* gametophytes with 2-celled marginal glandular hairs, the basal cell oblong in outline and the terminal cell inflated and globular—all the known species of *Diellia* and the present species of *Asplenium;* and with narrow 4-(2–5)-celled glandular hairs, the terminal gland-cell not so large in relation to the subtending cells as in the foregoing—the species of *Ceterach* and one Mexican species of *Asplenium.* The gametophytic fragments found on the rhizome of this fern, like the young sporophyte to be described below, provide therefore an unexpectedly detailed point of comparison in this study, remarkable for a species known only from three herbarium sheets.

The leaves of the juvenile sporophyte of *Asplenium leucostegioides* are known from several very young sterile fronds, and from the 8 somewhat older, fertile fronds of the type collection. They are unique in their large dimensions, another feature which suggests the genus *Diellia* but which contrasts with more typical rock-spleenworts. In *Diellia,* young leaf stages that show the first formation of pinnae (i.e., those which have either basal lobes or distinct pinnae in a single pair) average 16 mm. in length including stipe, and 13 mm. in breadth, in the genus taken as a whole. In such a rock-spleenwort as *Asplenium Trichomanes,* on the other hand, the fronds at this stage are less than half as large, averaging only 8.0 mm. in length, and 5.5 mm. in breadth. Although there are only 4 of the very young juvenile fronds of *A. leucostegioides* known at present, it is clear that they are unusually large—even larger than the average for *Diellia.* The two fronds which are simple are 17 and 21 mm. in total length, and 6 and 7 mm. in breadth, and have 5 and 8 vein terminations in the blade margin respectively. The one frond with a single pinna pair is 26 mm. long and 14 mm. broad, with 22 vein termini in the blade. This frond corresponds in size closely with the largest juvenile fronds known in *Diellia,* those of *D. falcata* and *D. laciniata.* The remaining sterile frond, which has 2 pinna pairs, is 3.7 cm. long and 1.5 cm. wide, with 33 vein termini. The apical "pinna"

in the pinnate juvenile fronds, including the 8 fertile ones, tends to be relatively large, just as in *Diellia* fronds of corresponding complexity. The fertile fronds of the type collection have an average of only 6.6 pinna pairs (5–8), a situation again similar to *Diellia* but contrasting with such spleenwort species as *Asplenium Trichomanes, A. normale,* and *A. monanthes.* In these three Asplenia under normal conditions 10 or more pinna pairs are usually produced before the fronds become soriferous; the leaf stages preceding sorus formation are apparently always smaller in size than in



FIGS. 54–57. *Asplenium leucostegioides,* glandular hairs on the lamina. FIGS. 54 & 55. Clarke specimen (GRAY). FIGS. 56 & 57. Baldwin specimen. FIG. 58. *A. leucostegioides, Clarke* (GRAY), an unusually long and jagged-margined indusium (not to scale). FIGS. 59–63. Discharged sporangial capsules of various species. FIG. 59. *A. leucostegioides, Clarke.* FIG. 60. *A. leucostegioides,* Baldwin. FIG. 61. *Diellia erecta,* Piiholo, Maui, *Wagner 5807.* FIG. 62. *A. Trichomanes,* Virginia, *Wagner 2023.* FIG. 63. *A. normale,* Haleakala, Maui *Wagner 5246.*

A. leucostegioides, and the figures given above for *A. Trichomanes* are representative of the group for the stage of pinna formation. Young sporophytes of *A. normale* (for example, *Elmer 11398,* Philippine Islands, GRAY) are similar in size to those of *A. Trichomanes,* and differ from the latter mainly in their more asymmetrical pinnae. The first few leaves produced on young plants of *A. normale* formed vegetatively at the tips of the rachises in the Hawaiian population of this species are of considerably more advanced structure than those on sporophytes produced by ordinary sexual reproduction; the size of frond stages of equal complexity whether the plants bearing them are produced sexually or asexually, however, pro-

duces a striking contrast with corresponding stages of *A. leucostegioides.* For example, an individual of *A. normale* which was vegetatively produced and which roughly corresponds in structure of fronds to the type specimen of *A. leucostegioides* (that is, has an average of 6.5 pinna pairs) may be compared with it as follows: fronds 10, all sterile, averaging 3.3 cm. in length, 0.7 cm. in breadth (*Mann & Brigham 186,* Oahu, GRAY) as compared with 8 fronds, all fertile, 6.5 cm. in length, and 2.1 cm. in breadth, of *A. leucostegioides.* Fronds of *A. normale* with 2 to 4 pinna pairs are usually less than 1 cm. long and 0.5 cm. in breadth. The size of early leaves in *A. monanthes* is practically the same, and may most easily be illustrated by the plants which are produced in the Hawaiian population by the proliferation that occurs at the base (rather than the tip) of the blade. *A. leucostegioides* thus resembles *Diellia* not only in respect to the large size of the early leaf stages but also in the appearance of sori when there are relatively few pinna pairs.

In conclusion it is evident that this species of *Asplenium* has numerous distinctive features combined as in *Diellia* and not as in its presumed relatives in *Asplenium.* The question, then, arises whether *Asplenium leucostegioides* may not indeed be an intergeneric hybrid. There is, however, little evidence for such an interpretation. On the contrary, *A. leucostegioides* has features not shown by the related spleenworts or by the genus *Diellia.* Its leaf axis is twisted so that it shows in mature fronds but little evidence of ridges on top. Sporangia, spores, and various other parts of the plant have considerably larger cell size than in any other members of the Aspleniaceae discussed here. That *A. leucostegioides* could be a hybrid combination which is polyploid might be suggested. But the fact remains that no known *Asplenium* of the Hawaiian Islands is suggested by its characters. The fern under discussion lacks such features of *A. normale* and *A. monanthes* as long, narrow sori; stalked or constricted pinna bases; grooved leaf axis; and others. The leaves are not conspicuously tufted as in these or in *A. Trichomanes,* nor does it show tendencies toward any of the other peculiarities of the latter familiar species of Hawaii. Most hybrids known in the Aspleniaceae have abnormal, abortive spores; examples of species hybrids which have been found to have abortive spores are *X A. Trudelli* and *X A. ebenoides.* One peculiar suspected hybrid *Diellia* has also been found which shows abortive spores. But the spores of the present species are seemingly normal, and the presence of a young sporophyte and gametophytes in the type collection suggests that this species is fertile. *Asplenium leucostegioides* might equally well be postulated as a sort of "reversionary" mutation, but I do not believe this to be the case. The combination of entirely free veins, asplenioid sori, twisted rachises, and large cell size, would seem to be an unreasonably large number of characters

to be involved in a single mutation. On the basis of present evidence, at least, the conclusion appears warranted that *A. leucostegioides* is a normal species.

The relationships of this fern, as judged by comparative morphology, possess unusual phylogenetic interest. The species described for the first time in detail here is obviously a member of the group of typical rock spleenworts as shown by the cross-sectional anatomy of its leaf axis, the characteristic laminar hairs, and other features. But it shows few of the specialized characters possessed by some others of the presumed members or derivatives of this group. Thus its scales are attached along a basal line, as is most common in this assemblage of ferns; they are not pseudopeltate in form (attached at a point and so strongly cordate that the margins overlap behind) as in *Ceterach officinarum*. The rhizome is erect, compact, and radial in construction. The sclerified, brittle leaf axis is darkly pigmented and lustrous, not green above the base of the stipe as in such a species as *Asplenium viride*. The frond is narrowly lanceolate with numerous pinnae, not lobed as in some species of *Ceterach*, nor simple as in *Schaffneria nigripes*, nor paucipinnate as in *Asplenium Ruta-muraria*. The outlines of the pinnae are typical in a general way of those of most spleenworts, not deeply dissected as in *A. formosum* nor extermely attenuate as in *A. septentrionale*. The veins are free, not reticulate as in *Camptosorus* or *Schaffneria*. The sori are straight and located unilaterally in a medial or supramedial position along the veins, unlike the "bent sori" of *Camptosorus* or those of *Diellia* which are uniquely oriented along the margin, with a special fertile vein. In spite of its thus relatively unspecialized evolutionary state within its group, this fern strongly suggests a prototype of the diversified and obviously rather highly evolved Hawaiian genus *Diellia*. To summarize the evidence, *A. leucostegioides* shares more than a dozen conspicuous features with the genus *Diellia* but not with its presumed immediate relatives in *Asplenium:* the general plant habit with robust stipes and few remaining dead rachises; the abundance and persistence of leaf-axis trichomes; the large relative size and structure of the rhizome scales; the sessile or adnate attachment of the pinnae, and their truncate basal margins; the straight or undulate laminar margins and absence of crenations or dentations; the short sorus, the indusium being about three times as long as broad; the darkly pigmented spores; the two-celled glandular hairs of the gametophyte; and the large size of the young sporophyte leaves, and the early appearance of sori, when there are only five pinna pairs.

This *Asplenium*, because of these peculiarities and its rarity, takes its place among the most unusual Hawaiian ferns. In its scarcity it is in a class with such unique endemics as *Pteris Lidgatei, Diellia Mannii, D. laciniata*, and *D. unisora*. Although of itself *Asplenium leucostegioides* would at first

sight appear to be merely another species of the familiar "black-stemmed" rock spleenworts, and was actually identified with common species of this group by several authorities, its points of resemblance to the unusual and diversified genus *Diellia* provide it with special significance. In fact, it is a fern with the real possibility of being a direct and perhaps little-modified descendant of the immediate ancestor of *Diellia*. The existence of such a plant expands even further, and fortifies, the now very considerable evidence that *Diellia* is neither a lindsaeoid nor davallioid genus, but is instead a member of the "superficial series" of leptosporangiate ferns, asplenioid in origin and related specifically to the group of rock spleenworts. The special phylogenetic interest which thus attaches to this plant will make highly desirable efforts to re-discover and study it in the field.

SUMMARY

1. Recent studies of the problematic Hawaiian fern genus *Diellia* indicate that it is neither lindsaeoid nor davallioid in affinity as had been previously believed. Present evidence indicates that this genus is instead a relative of the genus *Asplenium* and is derived by the evolution of marginal sori and secondary commissural veinlets specifically from the group of "black-stemmed" rock spleenworts typified by *A. Trichomanes*.

2. No individual member of the latter group, however, has heretofore been reported which suggests a specific prototype of *Diellia*. Described and discussed in detail for the first time here is a previously misinterpreted Hawaiian "black-stemmed" spleenwort, *Asplenium leucostegioides* Baker, known from only three collections made in 1879 on the island of Maui, an *Asplenium* which actually does suggest a prototype of *Diellia*.

3. This species may be distinguished from its presumed immediate relatives in *Asplenium* by the following ensemble of critical features, all of which it shares, however, with *Diellia:* robust stipes and early decay of old rachises; abundance and persistence of fibrils and glandular hairs along the rachis; large and conspicuous clathrate paleae; sessile or adnate pinnae; truncate basal margins of the pinnae; entire laminar margins which may be straight or undulate; much-reduced basal pinnae; short sori, with the indusia in full-sized fronds usually only three times as long as broad; darkly pigmented spores; 2-celled gametophytic hairs of characteristic structure; young sporophytes with early leaf stages of unusually large size, and appearance of sori when there are as few as 5 pinna pairs.

4. The present facts do not indicate that this fern is an intergeneric hybrid of *Asplenium* and *Diellia*, or that it is a mutation of *Diellia;* it appears to be a normal species. Of its features, the large cell sizes found in sporophytic and gametophytic tissues, and the somewhat twisted, apparently only slightly ridged rachises, seem to be somewhat unique. The inter-

esting phylogenetic implications of *Asplenium leucostegioides* make it desirable that efforts be made to re-locate and study this fern in nature.

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BULLETIN OF THE TORREY BOTANICAL CLUB

VOLL. 80, NO. 1, pp. 95–101 JANUARY, 1953

TORREYA

Clematis dioscoreifolia var. robusta (Carr.) Rehd. The writer has found this beautiful vine on two occasions, and suspects that it may eventually become established as part of our local flora growing without cultivation. Gray's Manual of Botany, 8th Edition, states that it is an occasional escape from cultivation, found from Massachusetts to Florida and Tennessee, and introduced from east Asia. On August 29th, 1951, the writer found the plant growing profusely along a section of a log fence adjacent to the Greenwich, Connecticut, railroad station. It grew with other tall weeds in a debris filled area, and had wound itself around the fence rails. There was apparently no garden from which it came in the immediate neighborhood. It was in flower on this date. On September 28th, 1952, the writer again found this vine in flower in Van Cortlandt Park, Bronx, New York City, growing along Mosholu Avenue just west of Jerome Avenue. It grew in a large bed of the cypress spurge (*Euphorbia Cyparissias* L.) where it seemed to be doing well in competition with this pernicious weed. There are no gardens in this location but Woodlawn Cemetery is about 500 feet east on the other side of Jerome Avenue.

From these few reports, the writer thinks that the plant may eventually run wild along walls in places where the soil is somewhat sandy as it was in both locations, and in competition with other well established plants. It may prove to be as hardy as our native *Clematis virginiana* L. to which it is closely related. It is a conspicuous plant in flower. The five leaflets are ovate and unlobed; the leaflet resembling the leaf of our wild yam; and the apex is pointed with a mucronate tip. The leaflets are smooth and somewhat coriacious. The inflorescence resembles that of *C. virginiana* L. with somewhat larger flowers. The writer has not seen its fruit.—WILLIAM RISSANEN.

Francis W. Pennell has been the subject of so many biographies in the news, and in scientific and botanical journals, that I believe it would be more fitting to offer at this time a somewhat personal appreciation. So with the barest outline of his life, I shall speak of him as I knew him.

Dr. Pennell was born Aug. 4, 1886 on a farm in West Chester County, Pennsylvania, attended the Friend's School in Westtown, took his B.A. in 1911 at the University of Pennsylvania, and his Ph.D. there in 1913. Under the guidance of Dr. John W. Macfarlane, head of the botany department there, he made some research along the Darwinian Theory of Evolution in a study of one of the genera of the Schophulariaceae. It was this work that gave him his slant in botanical study, and resulted in his becoming an international authority on the "Scrophs", as he familiarly called that family.

From 1914 to 1921 he was associate curator at the New York Botanical Garden, and since that time has been Curator of Plants at the Academy of Natural Sciences in Philadelphia. He was a member of the American Association for the Advancement of Science, The Botanical Society of America, American Plant Taxonomists, The Torrey Botanical Club (one time secretary-treasurer), Pennsylvania Botanical Society, and the Philadelphia Botanical Club. He was president of this last organization, and editor of its journal, Bartonia, since 1924. As its editor he promoted and personally wrote many articles on the botanical history of Philadelphia.

He made many botanical trips in the western United States and Mexico, and in South America to Colombia, Chile, and Peru, practically always for the collection and study of his "Scrophs". He had not yet begun the examination and study of his last collection in Peru in 1948.

I came to know Dr. Pennell about ten years ago when I persuaded him to publish in Bartonia my account of "The Cladoniae of Pennsylvania". At that time I knew very little about the preparation of manuscript for a scientific paper. Dr. Pennell was more than generous with his time in advising and assisting me. A few years later he again undertook the publication of my "Supplement to the Cladoniae of Pennsylvania",

although he confessed some bewilderment over the multiplicity of varieties and forms of the Cladoniae and the countless collections in my report.

He was always very generous of his time in assisting one and all who came to him with some real botanical problem, and had endless patience with the ignorance of beginners. He took time out of his precious hours on the Peruvian mountains to make a good collection of lichens for me, with abundant notes of locality, altitude, and habitat. It was a personal regret that I was not able to hand him my report on his collection, now appearing in the September (1952) issue of the Bryologist.

It was Dr. Pennell's invariable custom to call for me at lunch time during the 10 or 12 years I have been at the Academy. In this way I came to know him well,—not intimately, as I doubt he was intimate with any of his associates,—but in a very friendly way. All his interests, as revealed in his conversation, were centered in the botanical sciences. What was going on socially, politically, or economically, either locally or throughout the world, apparently was of little concern to him. Of the greater problems of life, religion, life and death, they too never appeared in our conversation.

The luncheon group might consist of two or ten, but generally Dr. Pennell led the conversation. It usually consisted of personal collecting experiences, his relations with the botanists of his wide acquaintance, of his problems with the "Scrophs", and his plans for future trips, study, and publication.

For his lunch he took sea food whenever it was served, was very fond of pies, and abhorred onions. As one brought up in a more economical day he counted his change.

His strongest point was also his weakest. The demands of his personal interest in the Scrophulariaceae were always in conflict with his curatorial duties, and usually the "Scrophs" won. Again, and knowing I speak "de mortuis", I could never determine whether it was his Quaker training or a personal trait that caused him to accept silently criticism, both of his work as curator and his devotion to his plants, which at times in my opinion was discourteous. I often wished he would make a defense of his position, and of the botanical department, as well as of his work.

About 1942 at his request I assumed the care of the long neglected Eckfeldt collection of lichens at the Academy. Although it was a very dirty task, and for the most part a thankless one, it proved to be the door through which I entered into the acquaintance of one who recognized his work, became absorbed in it, loved it, and died with it,—Dr. Francis W. Pennell.

Mrs. Anne W. Pennell, his wife, may be addressed at her home in Moylan, Delaware County, Pa.—W. L. DIX, Academy of Natural Sciences of Philadelphia.

BOOK REVIEWS

Families of Dicotyledons. By A. Gundersen. xviii + 237 pp. Illus. Chronica Botanica Co., Waltham, Mass. 1950. $4.75.

If Darwin could return today, he would undoubtedly experience deep satisfaction that the evolutionary concept which he developed with such meticulous care is accepted almost universally, and that many objections current in his day have vanished. Conversely, he would probably also be disappointed that, almost a century later, direct experimental evidence for the transformation of one species into another, especially among higher forms, is still largely lacking. Perhaps this is so, at least in part, because time can not be accelerated, even though the normal factors of the environment can be intensified. Conclusive experimental proof for the derivation of one family from another is at present nonexistent, and any system of classification will be subject to revision as knowledge increases. Dr. Gundersen therefore rightly cautions in this book on the dicotyledons that "the arrangement adopted is only tentative, not to be accepted but to be improved", and that "there remain many problems and uncertainties."

The "Engler" system has been in force now for some fifty-odd years. Although it has been exceedingly important in concepts of angiosperm interrelationships, it is generally admitted that modification is necessary, and various other plans have previously been published. Almost all the new manuals, however, even the best, still

essentially adhere to Engler's scheme, either for practical herbarium reasons, or for lack of a stable substitute, or because of innate botanical conservatism. It is especially gratifying, therefore, to see that Dr. Gundersen departs from this and begins the systematic treatment with the *Magnoliflorae*, including in the Magnoliales description of the Degeneriaceae. This volume represents the mature conclusions of a thoughtful lifetime of contact with these plants. Only by periodic and reflective assessment such as this can progress be made; only by the publication of such evaluations can the attainment of a true picture ultimately be achieved.

After a general discussion of the characters of dicotyledons and of historical notes, the systematic arrangement of 42 orders and 240 families is presented. These are considered in 10 main groups: *Magnoliflorae, Cistiflorae, Thea Group, Rosaeflorae, Ulmus Group, Malva Group, Geranium Group, Dianthiflorae, Jasminiflorae* and *Rubiflorae*. Six are designated *-florae* "to suggest that they may have a better claim than others to be considered as natural groups."

Each family is then delivered as a neat package containing the number of genera, and general descriptions of vegetative equipment, inflorescences, flowers, fruits and seeds. Representative and significant genera are cited. The illustrations accompanying many of the families are well chosen, clear, instructive and dignified. References to literature are included for the families, with emphasis on publications more recent than 1925. Chromosome numbers are listed with the orders. This portion of the book is packed with information; it will be especially helpful to students becoming acquainted with the families of flowering plants.

Those who study the book will notice that the Magnoliales are distinct from the Ranales; that groups with usually parietal placentation, including the Cucurbitaceae, are placed near the beginning; that the Salicaceae are put in the *Cistiflorae*, the Fagaceae and Betulaceae in the *Ulmus Group*, the Juglandaceae and Myricaceae in the *Geranium Group;* that the Ericales are in the *Thea Group;* that the Caryophyllales, Polygonales and Primulales are together in the *Dianthiflorae;* that the Umbellales, Rubiales and Asterales constitute the *Rubiflorae;* that the Campanulales and Asterales are separated, with the Campanulales in the *Jasminiflorae*. Exception may and undoubtedly will be taken to some of these points, but at least they offer food for thought.

The growing importance of allied fields to the understanding of angiosperm interrelationships is brought out in Part 1 of the book. This contains brief but potent accounts which serve as valuable introductions by C. A. Arnold on Fossil Dicotyledons, by O. Tippo on Wood Anatomy, by T. Just on Carpels and Ovules, by H. F. Copeland on Embryology, by J. H. Taylor on Cytotaxonomy, and by W. H. Camp on Plant Geography.

It is not inconceivable that, in the distant future, the key that unlocks the process of organic evolution may be found, and new forms may rapidly be induced to develop in all directions. Then interrelationships would take on an entirely new meaning, and the milestones in our appreciation of angiosperm affinities would stand out in bold relief. It is quite likely that the name of Dr. Gundersen will be found inscribed on one of those milestones.—Edwin B. Matzke, Department of Botany, Columbia University, New York, N. Y.

General Genetics. By Adrian M. Srb and Ray D. Owen. Illustrations by Evan Gillespie. 544 pages. W. H. Freeman and Company, San Francisco. 1952.

"General Genetics", the latest textbook in Genetics is a far cry from the earliest ones of the mid-twenties. This book is a good indication of how rapidly and how far this young science (dating from 1900) has advanced. The problems of earlier days have been solved and new ones, far more complex, involving considerable background knowledge in the closely allied subjects, cytology, biochemistry, physiology, morphogenesis, and practical agriculture, are posed. The answer to these problems lies in the experimental work being conducted by many workers in many places.

The authors have, in their own words, abandoned "anything like the systematic

historical approach to the subject''. One misses the old, familiar repetition of Mendel's work with the homely garden pea used to introduce the student to the simple monohybrid and dihybrid crosses. The garden pea has the advantage of being known to all students, city-bred as well as rural. Can as much be said in favor of ''frizzled fowls'', whether ''extreme frizzled'' or ''mild frizzled'', a favorite characteristic used by the authors, or for the cyanide content in white clover? On the whole, however, examples of inheritance are well chosen from widely separated areas in both plants and animals.

Realizing that differing amounts of time are allowed by various instructors for the presentation of this course, the authors have wisely subdivided their subject matter so that certain chapters, or portions thereof, may be omitted, as student needs demand.

The basic, or so-called ''classical'' genetics, is pretty well-covered in chapters 1–16. The subject matter is clearly presented so that one step leads to the next: the monohybrid cross, the dihybrid with some of its variations, followed by an interesting discussion of the influence of the environment on the expression of a given character. The next chapters deal with sex chromosomes and sex linkage, the chromosomes as the ''vehicles of inheritance''. Chapter 8 is a particularly good treatment of the variety of life cycles to be found in organisms. The choice of examples is admittedly a ''rather arbitrary'' one designed to be ''representative of large groups'', also ''that the organisms chosen be important objects of genetic research'', e.g., corn, *Sphaerocarpos*, *Neurospora*, and *Paramecium*. The following chapter deals with linkage and its allied phenomena of crossing-over, map making, interference. The only unvarying characteristic of life is that it varies, this variation is the very basis of its evolution. Succeeding chapters deal in turn with the sources of these variations: chromosome aberrations, changes in chromosome number, mutations. Extranuclear inheritance rates a chapter by itself. The complex phenomenon of sexuality is dealt with from all angles: chromosomes, genes, are all brought in in an attempt to help clarify the complicated problem of quantitative inheritance. This section of the book ends with a chapter devoted to two of the geneticists' most useful tools, inbreeding and selection.

Chapters 17, 18, 19 are given over to ''some of the details of genic effects that have emerged from recent investigations'' (page v). The biochemist makes his contribution to genetics when he attempts to explain the role of genes and enzymes in metabolism, but the question, ''*how* do genes control biochemical reactions'', is among the problems of the future. A few pertinent accepted facts concerning genes, ''the particles of heredity'', are summarized in chapter 19. This chapter concludes with a discussion of the human blood groups and the Rh factor with its puzzling problem, the nature of the ''gene'' at the Rh locus.

Chapters 20 and 21 deal with population genetics; 22 and 23 emphasize the application of genetic principles to plant and animal breeding respectively; while 24 ''summarizes aspects of genetics that relate to the welfare of the human individual'' (page vi).

The reference list found at the end of each chapter is a most extensive one; many of these are well beyond the average undergraduate both from the standpoint of time and subject matter. In some of their discussions on pertinent matters the authors are presuming a broader chemical foundation than many juniors and seniors have had time to acquire along with their other activities. However, as reference for source material for the student majoring in this field, the lists are excellent.

Good, thought-provoking problems which add greatly to the student's understanding of the subject matter accompany each chapter. The book is well illustrated.

''General Genetics'' is designed for an introductory course in genetics. To this reviewer the text is for the student who intends to major in the field rather than for one seeking a general knowledge of the subject.—HETTIE M. CHUTE, New Jersey College for Women, Rutgers University, The State University of New Jersey.

FIELD TRIP REPORTS

May 10–11, 1952. Camp Thendara, Interstate Park, N. Y. This was to have been a joint outing with TBC the guest of the Green Mountain Club, New York Section, but no botanists were reported. The bird counting members of GMC made their usual good

showing and complete report. One of our pleasantest, and certainly one of our cheapest spring trips, we cannot expect to be invited unless more interest is shown.—J.A.S.

May 24. Van Hiseville to Island Beach, Ocean County, N. J. Several stops in the pine area yielded the expected plants in bloom and otherwise. A particularly striking stand of *Lupinus perennis* was seen at Van Hiseville. *Viola primulifolia, V. pedata,* and *V. fimbriatula* were also shown in unusual abundance, but mostly past flowering, along the route. At Island Beach the usual maritime flora in unusual and undisturbed profusion was noted with perhaps most attention devoted to oak hybrids, large specimens of Spanish oak and the holly trees. Attendance 13. Leader, Helen Camp.

July 5–6. Salisbury, Conn. In spite of the hot and dry weather, the joint meeting with the Connecticut Botanical Society was a successful one. The summit of Mt. Riga or Bald Mountain was first botanized, the Catskill Mts. plainly visible in the west and northwest. The highlight of the afternoon was a trip into a tamarack-black spruce bog known as Bingham Pond. The dwarf mistletoe was found. A third stop was in Sages Ravine, now only accessible by the Appalachian Trail. The lower part of the ravine near Twin Falls is very difficult to traverse because of huge hemlock tree blowdowns caused by the 1950 November gale.

On Sunday the attendance dropped from 25 to 15. (The TBC members were 10 each day.) Previous plans were changed and the party explored an old iron ore bed now covered with very green-colored water. This pond lies on the east side of Bear Mtn., Conn., about a mile south of the Conn.–Mass. line. Limestone and schist contact in this area. So many Habenaria (in fruit) were found in this area that the TBC members said a field meeting there next spring was a must. After exploring a swamp in the afternoon the party disbanded. Lists were kept and totaled over 75 species in the two days. Mosses and lichens were well represented. Most uncommon species: *Amblystegium fluitans* and *Hydrothyria venosa.* Leader, Walter P. Green.

Aug. 17. Sims Place, Burlington Co., N. J. We saw the usual exotic trees in Point Pleasant before going to the pine barrens. At Sims Place the *Habenaria integra* was at its best and seems to be holding its own. The *H. cristata* that formerly grew in the vicinity was not found on this trip. *Aspidium simulatum* was found growing in abundance just north of Silverton. This fern is described by Stone (Plants of Southern New Jersey) as "apparently restricted to boggy spots or cedar swamps on the edge of the pine barrens." Attendance 5. Leader, V. L. Frazee.

Aug. 16–17. Cape May, N. J. Every plant we searched for was located except the square-stemmed sabatia (*Sabatia angularis*) which this year at least appears to have given up the struggle on the Cape May salt marsh. A small patch of the blue mist flower (*Eupatorium coelestinum*) persists despite crowding by other species. It certainly has declined since Witmer Stone's day, for he states in his Flora of Southern New Jersey that the salt marsh between Cape May and Cape May Point was covered with the plant in proper season.

Bennett Bog had about 8 inches to 10 inches of water in most parts of it during the summer season and many of the plants responded by augmenting their numbers. *Spiranthes praecox* presented hundreds of blooms as did *Habenaria nivea* and *Xyris fimbriata. Sclerolepis uniflora* has not been as abundant since the 1947 season.

On the salt marshes near Stone Harbor a beautiful adult yellow-crowned night heron was observed at leisure by the group and the Hudsonian curlew fed in the *Spartina* grass nearby. The nightly influx of egrets and herons into the Stone Harbor roost was observed among which several Louisiana herons were noted. The mayor of Stone Harbor was on hand to greet our group as well as Mr. and Mrs. Shea and Miss Thomas of the Stone Harbor Bird Club. Attendance 16. Leader, David Fables.

Aug. 23. Wolf's Pond Park, Staten Island. Persimmon, bitter-sweet, and the two Cassias were most interesting of the upland plants observed. Cardinal flower and marsh-

mallow in full bloom at the pond's edge were likewise attractive. Equally so was the quillwort (*Isoetes engelmanni*) which was found in the brook. There was sea-rocket, glasswort, and sand-spur (*Cenchrus tribuloides*) along the beach where sanderlings, semi-palmated plover, and killdeer were also seen. Attendance 6. Leader, Mathilde P. Weingartner.

Sept. 14. Cheesequake State Park, N. J. This was our 18th official trip to the park in its thirteen years of existence. During that time our natural history survey of the area has recorded 24 algae, 98 fungi, 37 lichens, 19 bryophytes, 19 ferns, etc., 412 herbs, 68 shrubs, and 45 tree species. We have a combined bird list of 102 species. Our greatest discovery on this trip was a grievous one. The Garden State Parkway will cross the area. The tragedy of this can hardly be exaggerated. The park will be bisected, the developed part cut almost equally in half. The right-of-way will remove many acres, mostly upland. Our parks operate on a very meager budget for development and maintenance. The facilities at Cheesequake are only there because of a pubilc works project during the depression. Nearly all of the parkway alignment is in this developed portion. The necessary grading will change the drainage and threaten erosion. In addition to the usual forest trees, shrubs, and ground plants which are richly represented, this swath will remove countless dogwoods, mountain laurel, bittersweet, and lady's slippers. A stand of *Smilacina stellata*, discovered on this trip will be lost as will the only known basswood trees of the park. No one would think of permitting these plants to be dug up by the best intentioned visitor to the park, but the Highway Department can bulldoze them out with impunity. By what right can one department of our state government so impose upon another? There is no question of the need and use of this recreation area. There is no question but that the parkway could have avoided it.—J. A. S. Attendance 16. Leader, L. E. Hand.

Sept. 20. Manasquan to Beach Haven, N. J. The dried fill at Manasquan Inlet discouraged the golden plover from visiting it this autumn and we did not record the species. Even at Beach Haven Inlet bird life was at a comparatievly low ebb. A caspian tern was the outstanding "find" of the day, although there were still many black skimmers to add beauty to the scene. Black-bellied plovers were the most common shore birds. A small diamond-backed terrapin was picked up on the beach front. There were still hundreds of blossoms of *Sabatia stellaris* and *Pluchea camphorata* as well as scattered blooms of *Limonium carolinianum*. Attendance 8. Leader David Fables.

Sept. 27. Staten Island, N. Y. Approximately 36 species of grasses were identified by the leader and several species of hybrid oaks were described by Mr. Lee Ellison. The oaks included *Quercus heterophylla*, (a cross between blackjack and red oak), and *Quercus rudkini* (a cross between willow oak and blackjack oak), hybrid oaks with strong indications of Spanish oak were also found in Tottenville, near the south end of the Island.

A number of interesting salt marsh plants were also found on the trip including *Aster tenuifolius* and *Aster subulatus*, *Prunus maritima*, *Baccharis halimifolia*, *Iva frutescens*, *Pluchea camphorata*, and three species of *Spartina*. A small stand of *Ipomoea pandurata* (man of the earth) was found in the sandy soil of the Tottenville area. This is the only known growth of this plant on Staten Island.

The entire trip covered seashore, salt marsh, upland field and sandy woods areas. No fresh water marshes were visited. Attendance 10. Leader, Robert C. Meyer.

Sept. 28, New Brunswick, N. J. A visit was made to the Raritan River flood plain at a site which is called Raritan Landing on the geological maps of New Jersey. An ecological study is being made there. A walk upstream along the river's edge was followed by visiting three abandoned fields, adjacent to the river bank, which are in different stages of old field succession. Basal diameters were taken of a sycamore near the river and a dead pin oak near a field. *Platanus occidentalis* measured 58 inches and *Quercus palustris* measured 51 inches. Five members of the Newark Nature Club were with us as guests. Attendance 18. Leader, Warren A. Wistendahl.

Note

Camillo Karl Schneider, eminent German dendrologist, died recently (5 January 1951) in Berlin in his 75th year. Under the Nazi regime, the war, and subsequent disorders, the life of an elderly scientist was not easy. Dr. Schneider spent his last years in severe privation.

He left behind him a large library, mainly of horticultural books but containing also many botanical works. This library is for sale, either as a whole or in part, by his widow. Frau Schneider has furnished a typewritten list which is available at the library of The New York Botanical Garden in the care of the undersigned. It includes more than 1000 titles, some being runs of periodicals consisting of many volumes. Prices would presumably by the subject of arrangement between Frau Schneider and the purchaser.—H. W. Rickett.

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INDEX TO AMERICAN BOTANICAL LITERATURE

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TAXONOMY, PHYLOGENY AND FLORISTICS

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